A TAXONOMIC STUDY OF THE *BURKHOLDERIA CEPACIA* COMPLEX: AN ANALYSIS OF GENOTYPIC, PHENOTYPIC AND SUSCEPTIBILITY CHARACTERISTICS.

KIRSTI ANNE MORRIS

A thesis submitted in partial fulfilment of the requirements of the University of Northumbria at Newcastle for the degree of Doctor of Philosophy

March 2004

CONTENTS

FIGURES

TABLES

APPENDICES

Acknowledgements

I would like to thank the University of Northumbria at Newcastle and The Freeman Hospital for supporting this research project. I am indebted to Dr John Perry for providing me with the opportunity to carry out this research and for his expert supervision and encouragement throughout. I am also extremely grateful to Dr Gary Black for his continual advice and support throughout the entire course of my research. I would also like to express my gratitude to my colleagues and friends within the Microbiology Department at the Freeman Hospital and at Northumbria University for their continued support. My heartfelt thanks go to my parents, who have shown me great support and encouragement throughout my academic years. Finally, I am eternally indebted to my husband Jason, whose constant encouragement and emotional support throughout the production of this thesis contributed significantly to its completion.

Declaration

This thesis records the results of experiments conducted by myself in the Department of Applied and Molecular Sciences, University of Northumbria, and the Department of Microbiology, Freeman Hospital, Newcastle upon Tyne, under the supervision of Dr. G. Black and Dr. J. D. Perry between November 2000 and March 2004. It is of my own composition and has not previously been submitted in part, or in whole, for a higher degree.

Abstract

The development of novel molecular tools has provided the scientific community with quick, easy, and scientifically sound ways of identifying individual strains belonging to the *Burkholderia cepacia* complex (Bcc). Bcc strains isolated from the sputum of 44 patients attending the Freeman Hospital Cardiopulmonary Transplant Unit were genotyped using *recA* PCR-RFLP analysis, and a clonality study performed using PFGE analysis. It was found that *B. cenocepacia* and *B. multivorans* were the predominant colonizing strains in these patients, and that infection with the ET-12 epidemic clone was the most prevalent strain amongst *B. cenocepacia*- infected patients. It was also found that pre-transplant strains remained responsible for post-transplant infections.

Phenotypic methods for the identification of Bcc strains and closely related organisms have been difficult to develop. A collection of 493 strains including *Burkholderia cepacia* (genomovars I-IX), *Pseudomonas aeruginosa*, and other closely related organisms, were investigated for their abilities to produce a wide range of peptidases, glycosidases, esterases and other miscellaneous enzymes using both chromogenic and fluorogenic substrates. The 312 Bcc strains within the collection were also screened for their capacity to oxidise a number of carbohydrates. The heterogeneous nature of all nine Bcc species was confirmed by this study, as was the close phenotypic relationship of *B. cepacia* and *B. cenocepacia*. Some substrates, however, were shown to have some taxonomic utility for the differentiation of species within the Bcc and also for closely related organisms. Metabolic activities that showed diagnostic potential included production of ß-ribosidase, ß-xylosidase and ß-glucosidase, as well as oxidation of cellobiose, maltose and trehalose. Screening for palmitate esterase and α or ß-trypsin production was useful for the differentiation of *Pandoraea* sp. and *Ralstonia* picketti respectively.

CF infections caused by *P. aeruginosa* and Bcc strains, are most successfully treated using two or three drug combinations. A number of cell wall-acting antibiotics were tested in combination with the phosphonopeptide alafosfalin for synergistic effects against Bcc and *P. aeruginosa* strains. Alafosfalin was most effective in combination with ceftazidime against Bcc strains, and in combination with tobramycin and ceftazidime as a triple combination against *P. aeruginosa*.

CHAPTER 1

Introduction

Cystic fibrosis (CF) is the most commonly inherited lethal disorder of Caucasian populations, with an incidence of approximately 1 in 2500 live births and a carrier frequency of 1 in 25 (Bye *et al.*, 1994). The susceptibility of patients with CF to pulmonary infection has been recognised since the earliest descriptions of the disease in the 1940s and 50s (Anon, 1952). Typical CF respiratory pathogens include *Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae* and species belonging to the taxonomic group known as the *Burkholderia cepacia* complex (Bcc). Differentiation between members of the Bcc has proved challenging due to their phenotypic similarity. Although molecular techniques are now established which can identify the individual species of the Bcc, investigation into a purely phenotypic means of identification is still warranted.

Due to the recent and ongoing reclassification of the Bcc, terminology that will be used to refer to the members of the Bcc throughout this thesis must be described. The name *B. cepacia* will relate only to *Burkholderia cepacia* genomovar I. A large number of previous reports regarding these organisms were published before the recognition of the complicated taxonomic relationships between the different members of the Bcc, or choose to ignore the recent reclassifications; it is therefore unclear to which species the isolates described would belong. For that reason, when such literature is cited, the name "*B. cepacia*" will be shown in double quotes.

General description of "*B. cepacia*"

"*B. cepacia*" is a slender rod with peritrichous flagella that accumulates poly-ß-hydroxybutyrate and so stains irregularly in the Gram stain reaction. The rods are 1.5 µm long and 0.5-1.0 µm wide (Gilligan *et al.*, 1995). It is aerobic and grows well on nutrient agar but often prefers temperatures of 25-35 ^{O}C for 48 hrs for optimal growth. Most strains grow at 41^{O}C but not at 43 ^{O}C or 4 ^{O}C. Cultures on blood agar often become non-viable after 3-4 days and survival on refrigerated slopes is poor. Colonies on nutrient agar are opaque; those of some strains are greyish white, but others are at first yellowish and later take on an intense reddish purple colour due to the formation of a non-diffusible phenazine pigment (Holmes and Howard, 1994). This yellow pigment is best demonstrated on iron-containing media such as triple sugar iron agar. "*B. cepacia*" is exceptionally versatile nutritionally and is able to use many simple organic compounds as sole sources of carbon and energy for growth (Palleroni, 1984). Strains can also multiply in distilled water, dilute disinfectants, and can utilise penicillin G (Jayaswal *et al.*, 1993).

The genome of "*B. cepacia*" (strain 17616) is constituted by 3 large circular replicons of 3.4, 2.5 and 0.9 Mbp, which, in addition to a megaplasmid previously identified, gives a total of 7 Mbp, a large size in accordance with the nutritional and metabolic versatility of this species (Cheng and Lessie, 1994). The genome is significantly larger than that of *P. aeruginosa* (5.8 Mbp).

At least 3 serological typing systems have been described independently for the grouping of "*B. cepacia*" isolates (Wilkinson and Pitt, 1995), but the scheme of Werneburg and

Monteil, (1989) has probably been the most widely used. This differentiates the species into 9 O and 7 H types with typability of 98 % and 43 % respectively for isolates from the environment and nosocomial infections; typability rates fall significantly with CF isolates.

Taxonomic aspects and history

The Burkholderia genus

"*B. cepacia*" owes its present epithet to the description by Burkholder (1950) of a bacterium responsible for rot in onion bulbs, originally designated *Pseudomonas cepacia.* Other names that were assigned included eugenic oxidisers group 1, *Pseudomonas kingii,* and *Pseudomonas multivorans* (Stanier *et al.*, 1966; Jonsson *et al*, 1970), but studies clearly showed that these could be considered as synonymous names of *P. cepacia* and that the name *P. cepacia* had priority (Ballard *et al*, 1970; Snell *et al.*, 1972). In 1992, *P. cepacia* and six other species belonging to rRNA group II of the genus *Pseudomonas* (*Pseudononas solanacearum, Pseudomonas pickettii, Pseudomonas gladioli, Pseudomonas mallei, Pseudomonas pseudomallei and Pseudomonas caryophylli*) were transferred to the new genus *Burkholderia* (Yabuuchi *et al.,* 1992). Since the genus name was first assigned, the taxonomy of the genus *Burkholderia* has undergone considerable review and the genus now includes over 30 species occupying remarkably diverse ecological niches, ranging from contaminated soils to the respiratory tract of humans (Coenye and Vandamme, 2003).

Burkholderia cepacia complex (Bcc)

From the mid-1990s onwards, several researchers noted that there was a marked heterogeneity among "*B. cepacia*" strains isolated from different ecological niches. These strains were tentatively classified as "*B. cepacia*" using a wide range of techniques (Bevivino *et al.,* 1994; Butler *et al.,* 1995; Gillis *et al.*, 1995; Tabacchioni *et al.*, 1995). The heterogeneity among "*B. cepacia*" isolates made correct identification problematic, and evaluation of the techniques used showed that they were lacking in sensitivity and/or specificity (Coenye *et al.*, 2001a). The remarkable diversity among presumed "*B. cepacia*" strains and the lack of reliable identification schemes led Vandamme *et al.* (1997) to a polyphasic taxonomic study that demonstrated that presumed "*B. cepacia*" strains isolated from CF patients and other sources were a heterogeneous group of at least five genetically different groups or genomovars collectively known as the *Burkholderia cepacia* complex (Bcc) (Mahenthiralingam *et al.*, 2000a). The term genomovar was introduced to denote phenotypically similar but genotypically distinct groups of strains that were previously referred to by a variety of different terms including genomic species, genomic groups, genospecies, or genomospecies (Vandamme *et al.*, 1997). Determination of the genomovar status of Bcc strains was based on a polyphasic taxonomic approach which utilised phenotypic tests such as whole-cell protein profile analysis and genotypic tests such as DNA-DNA hybridisation (Vandamme *et al.*, 1996, 1997). These genomovars shared a low level of DNA hybridisation and therefore represent distinct species for which an official binomial name was not proposed pending the availability of differential diagnostic tests (Vandamme *et al.*, 1997).

B. cepacia genomovar V was identified as the previously described species *B. vietnamiensis* (Gillis *et al.*, 1995), and the name *B. multivorans* was proposed for the genomic species formerly known as *B. cepacia* genomovar II. The remaining groups were referred to as *B. cepacia* genomovars I, III and IV. Since *B. cepacia* genomovar I contains the type strain, it retains the formal binomial *B. cepacia*.

Phylogenetic analysis of the *recA* gene revealed that strains belonging to *B. cepacia* genomovar III could be further split into two distinct *recA* cluster groups designated III-A and III-B (Mahenthiralingam *et al.*, 2000a). DNA-DNA hybridisation experiments between strains representing *B. cepacia* genomovar III *recA* lineages III-A and III-B reinforced the classification of both phylogenetic subgroups as a single genospecies, distinct from *B. cepacia* (genomovar I). While the majority of *recA* III-A strains examined have all been implicated in instances of patient-patient spread (Mahenthiralingam *et al.*, 2000a), the taxonomic and clinical relevance of the division of genomovar III into III-A and III-B is currently not known.

Following a thorough investigation of the phenotypic and genotypic characteristics of *B. cepacia* genomovar IV strains (Vandamme *et al.*, 2000), it became obvious that this organism could be differentiated from all other members of the Bcc, and it was formally classified as *B. stabilis*. Subsequent polyphasic taxonomic studies identified two more members of the Bcc. In 2001, visual comparison of protein profiles indicated that a set of 18 "*B. cepacia*" strains isolated from CF patients in the USA represented a new member of the Bcc, designated *B. dolosa* (*B. cepacia* genomovar VI) (Coenye *et al.*, 2001b). *B. cepacia* genomovar VI contains strains isolated from CF patients in the United States

the United Kingdom. This organism could be differentiated phenotypically from all members of the Bcc except *B. multivorans*. Also in 2001, Coenye *et al.* carried out a polyphasic study on 19 "*B. cepacia*" isolates from the environment and CF patients (Coenye *et al.*, 2001c). The polyphasic taxonomic data showed that the strains represented a new member of the Bcc (designated genomovar VII), for which the name *B. ambifaria* was proposed. Coenye *et al.* 2001c reported that all the *B. ambifaria* isolates investigated formed a single homogeneous cluster and could be differentiated easily from other *Burkholderia* species and genomovars. The phylogenetic allocation and intermediate DNA-DNA binding values towards members of the Bcc indicated unambiguously that *B. ambifaria* represented a seventh genomovar within the Bcc.

A more recent study in 2002 (Vandamme *et al.*, 2002) described a group of 19 atypical "*B. cepacia*" organisms that could not be allocated to one of the then current seven members of this species complex. The name *Burkholderia anthina* sp. nov. was proposed to accommodate this eighth genomovar within the Bcc. Petrucca *et al.* (2003) reported the first case of multiple patient colonisations by *B. anthina* in a CF centre. An evaluation of published DNA-DNA hybridisation and 16S rDNA sequence data for *B. pyrrocinia*, a soil bacterium described in the 1960s (Imanaka *et al.*, 1965; Vandamme *et al.*, 1997; Viallard *et al.*, 1998), revealed hybridisation and similarity levels as reported between other Bcc bacteria. This was confirmed by analysis of the *recA* gene sequence which again revealed values similar to those observed between Bcc bacteria (Vandamme *et al.*, 2002). This was also substantiated by the results of the *recA* restriction fragment length polymorphism (RFLP) technique, as the primers used for the initial amplification of the *recA* gene were chosen such that they were specific for Bcc bacteria (Mahenthiralingam

et al., 2000a). Altogether, these data indicate that *B. pyrrocinia* should be considered a ninth genomovar within the Bcc.

A formal classification of *B. cepacia* genomovar III encompassing the *recA* lineages III-A and III-B, and the new *recA* lineages III-C and III-D, as *B. cenocepacia* sp. nov has been proposed (Vandamme *et al.*, 2003). All nine Bcc genomovars separate into distinct arms of the phylogenetic tree (Mahenthiralingam *et al.*, 2002).

Within the Bcc, representatives of different species all share a high degree of 16S rDNA (98 - 100 %) and *recA* (94 - 95 %) sequence similarity, and moderate levels (30 – 60 %) of DNA-DNA hybridisation (Vandamme *et al.,* 1997, 2000; Mahenthiralingam *et al.*, 2000a; Coenye *et al.*, 2001a, 2001b). Isolates from cystic fibrosis patients from different geographical regions and from novel environmental niches are currently being examined to continue to explore the biodiversity of this organism. The results from such studies challenge newly developed molecular identification approaches (Bauernfeind *et al.*, 1999; LiPuma, 1999; LiPuma *et al.*, 1999; Segonds *et al.*, 1999; Brisse *et al.*, 2000) by the discovery of novel "atypical" isolates that do not fit into the current classification system. For example, *Burkholderia ubonensis* is a recently described soil bacterium which is thought to be potentially a tenth genomovar but its relationship to the various Bcc genomovars requires further study (Vermis *et al.*, 2002).

Clinical Significance of *Burkholderia* sp.

Although most species in the genus *Burkholderia* are not pathogenic for healthy persons, a few are capable of causing severe, life threatening infection (Coenye and Vandamme, 2003). *B. mallei* and *B. pseudomallei*, for example, are the causative agents of glanders and melioidosis respectively. Interest in these species has increased recently owing to their potential for use as agents of bioterrorism (Coenye and LiPuma, 2003). The Bcc, however, seems to be the main pathogenic group of organisms within this genus.

"*B. cepacia*" can colonise a variety of moist environmental surfaces and is commonly associated with nosocomial infections (Martone *et al.*, 1987; Isles *et al.*, 1984; Govan *et al.*, 1993; Smith *et al.*, 1993). Infections caused by this organism include respiratory tract infections in patients with CF or chronic granulomatous disease; urinary tract infections in catheterised patients; septicaemia, particularly in patients with contaminated intravascular catheters; and other opportunistic infections (Murray *et al.*, 1998). "*B. cepacia*" infections in immunocompetent patients occur only sporadically, but cases of pseudoepidemics and nosocomial infections, often caused by contaminated disinfectant and anesthetic solutions, have been reported (van Laer *et al.*, 1998). With the exception of pulmonary infections, "*B. cepacia*" has a relatively low level of virulence, and infections with the organism do not commonly result in death (Nelson *et al.*, 1994). The major impact of "*B. cepacia*" infection has undoubtedly been in CF patients, in whom it was first recognised more than 20 years ago (Rosenstein and Hall, 1980; Isles *et al.*, 1984).

Cystic Fibrosis (CF)

In 1997, the median life expectancy for individuals with CF was 31.5 years in the UK and it has been suggested that those born today can expect to live well into their mid 40s (Duff, 2002). The CF gene has been identified, cloned and sequenced, and the structure of its protein product analysed (Riordan *et al.*, 1989). The gene, on the long arm of chromosome 7, is 250 Kb long, contains 27 exons, and encodes for a transmembrane protein of 1480 amino acids known as the cystic fibrosis transmembrane regulator (CFTR). Since the identification of the gene, more than one hundred gene mutations have been discovered. CFTR functions as a chloride ion channel protein (Drumm *et al.*, 1990). It is a protein that is thought to have a role in ion transport, mucus rheology, inflammation and bacterial adherence (Jaffe and Bush, 2001). In most CF populations approximately 70 % of individuals have a deletion of a single codon in one of the nucleotide-binding folds of CFTR gene leading to the deletion of phenylalanine at position 508 (ΔF - 508) (Riordan *et al.*, 1989). Persons homozygous for mutant alleles of the CF gene have severe defects in chloride ion transport.

The ineffective chloride transport due to CFTR lesions causes hyposecretion of electrolytes and water in lower airways and hyperabsorption of electrolytes and water in central airways. As a result, individuals with the condition have a characteristically salty sweat. Under these conditions of reduced hydration levels, mucociliary clearance is impaired by the viscid dehydrated nature of airway mucus and therefore may not be as efficient as normal in removing the bacteria and other pathogens trapped in the mucous blanket (Koch and Hoiby, 1993). This results in increased susceptibility to persistent

microbial colonization with intermittent episodes of debilitating, and ultimately fatal, infection. In adults, more than 95 % of the deaths from this condition result from a respiratory tract infection or a related complication (Penketh *et al.*, 1987).

Microbiology of CF

Before the advent of reliable antibiotic therapy most CF patients died in early childhood. Even though today the prognosis has improved such that most patients can expect to survive to adulthood, most patients eventually succumb to the overwhelming sequelae of repeated pulmonary exacerbations arising from persistent *P. aeruginosa* colonization, typically over a period of 10 or 20 years (Govan and Deretic, 1996). In general such infections are localised in the lungs, in particular the major and minor airways, rather than the alveoli. Infections at non-pulmonary sites are rare. Chronic microbial colonisation of the lower respiratory tract, leading to exacerbations of pulmonary infection, is responsible for much of the morbidity and mortality of CF (Bye *et al.*, 1994). Repeated infections further aggravate the condition resulting in a vicious cycle of chronic respiratory infection and inflammation. As a result, recurring episodes of pulmonary exacerbations cause irreversible tissue damage leading to respiratory failure and death (Davidson *et al.*, 1995).

In microbial pathogenesis, there are few more striking examples of *in vivo* microbial adaptation than the asymptomatic colonisation of CF lungs by typical non-mucoid *P. aeruginosa* strains and the subsequent emergence of mucoid forms during chronic debilitating pulmonary infection. The first descriptions of an association between mucoid

P. aeruginosa strains and chronic pulmonary colonisation in patients with CF appeared in the 1960s (Iacocca *et al.*, 1963; Doggett *et al.*, 1964). In their seminal studies, Doggett and colleagues reported that such strains could be isolated in up to 90 % of CF patients colonised with *P. aeruginosa*; in contrast, they were rarely cultured from other infections in humans, plants, or animals or from the wide range of environmental habitats associated with this ubiquitous organism. At this time, evidence suggested that in patients with CF, primary asymptomatic colonisation occurred with non-mucoid strains but that pulmonary deterioration followed later with the conversion to mucoid forms and the development of antipseudomonal antibodies (Burns and May, 1968; Diaz *et al.*, 1970). Epidemiological data obtained from pyocin typing and RFLP analysis have provided convincing evidence that unrelated CF patients are usually colonised with different strains and that once colonised, individual patients harbor the same strain for long periods (Godard *et al.*, 1993; Romling *et al.*, 1994).

Although pathogens most commonly isolated from the respiratory tract of CF patients are *P.aeruginosa S. aureus* and *H. influenzae* (Gilligan, 1999), other glucose non-fermenters, like *Stenotrophomonas maltophilia, Alcaligenes xylosoxidans, R. pickettii* and *B. gladioli*, can frequently be found as well, however, their role in the decline of pulmonary function is unclear (Christenson *et al.*, 1989, Gilligan, 1991). Susceptibility to a particular pathogen tends to be age related with *S. aureus* appearing in infancy, followed by *H. influenzae* in the early years, and *P. aeruginosa* in adolescence. Within the last 20 years, "*B. cepacia*" has been increasingly isolated from the sputum of CF individuals (Isles *et al.*, 1984; LiPuma, 1998a, 1998b). The first reports of the recovery of "*B. cepacia*" from sputum culture from significant numbers of individuals with CF in

Philadelphia and Palo Alto appeared in the late 1970s. A report by Isles *et al.* (1984) described the clinical significance of "*B. cepacia*" colonisation and infection in the Toronto centre, and also in the USA (Thomassen *et al.*, 1985). The prevalence of "*B. cepacia*" infection ranges from 2 % to 13 % in CF patients (Burns and Saiman, 2000; Mahenthiralingam *et al.*, 2002). Risk factors for the acquisition of "*B cepacia*" include greater severity of underlying CF, increasing age, having a sibling colonised with "*B cepacia*", and previous hospitalisations. In addition to documenting a steadily increasing prevalence of "*B. cepacia*" colonisation during the previous two decades, these authors described a syndrome of severe progressive respiratory failure. This is characterised by a necrotising pneumonia with associated bacteraemia which leads to a rapid and frequently fatal clinical decline, often in those with previously mild disease, that occurred in several patients (in about 20 % of all infected CF patients; Govan *et al.*, 1996). This condition has been named "cepacia syndrome".

Although all genomovars of the Bcc are capable of airway infection in CF patients, the majority of isolates are *B. cenocepacia* (>50% of CF infections) (Mahenthiralingam *et al.*, 2002) and to a lesser extent *B. multivorans* and *B. vietnamiensis*, with *B. stabilis* and *B. cepacia* (genomovar I) representing minor components (LiPuma, 1999; LiPuma *et al.*, 1999). Most cases of "cepacia syndrome" are now thought to be due to *B. cenocepacia* infection (Speert *et al.*, 2002). The observation that "*B. cepacia*" comprises a number of distinct species may help to explain why a variable clinical course has frequently been noted in those individuals infected with this organism (Frangolias *et al.*, 1999). Retrospective analysis of epidemiological and genotypic data suggests that strains of *B. dolosa* have been involved in chronic colonisation of CF patients and have been spread

from person to person, although its significance in morbidity and mortality is unclear (Coenye *et al.*, 2001b; Biddick *et al.*, 2003). Although *B. ambifaria* has been isolated from CF patients, again the potential pathogenic mechanisms are little understood. Most of the present strains of *B. anthina* have been isolated from the rhizosphere soil of garden flowers and houseplants, but have been isolated from sputum samples of CF patients (Vandamme *et al.*, 2002). The presence of these bacteria in the soil of various flowers and green plants suggests that this can serve as a reservoir for infections. However, during the past years, several thousands of CF-related isolates have been stored and examined in various national reference or referral centres, and thus far only a small number of human *B. anthina* isolates have been collected (Vandamme *et al.*, 2002).

Bcc and lung transplantation

Currently, the only available treatment for CF patients with advanced pulmonary disease is lung transplantation. The number of CF patients undergoing lung transplantation has risen over the past decade, because of a clear cut survival benefit (De Soyza and Corris, 2003). Such treatment offers significantly longevity benefits with 50 % of recipients enjoying a good quality of life for five or more years post-transplant (Hosenpud *et al.*, 1999). However, "*B. cepacia*" has emerged as an important pathogen in patients with CF undergoing lung transplantation. The outcome of patients with advanced CF undergoing lung transplantation is adversely affected in those colonised with "*B. cepacia*" with mortality rates of up to 80 % reported (Snell *et al.*, 1993). One year survival rates in patients with CF undergoing lung transplantation are generally less than 70 % (Husain *et al.*, 2002). Many transplant units therefore do not operate on such patients as they are at

risk of developing post-transplant "cepacia syndrome" and death. More recent studies have shown that of patients infected pre-operatively with Bcc, those infected with *B. cenocepacia* are at the highest risk of Bcc-related mortality (Aris *et al.*, 2001; De Soyza *et al.*, 2003, De Soyza and Corris, 2003).

Transmissibility

Clustering of new cases in some centres and the decrease of colonisation of new patients following segregation of colonised and non-colonised patients in other centres suggested that "*B. cepacia*" could be transmitted between CF patients (Martone *et al.*, 1987). This was confirmed by several studies (LiPuma *et al.*, 1990, 1994, 1998b; Millar-Jones *et al.*, 1992; Govan *et al.*, 1993; Biddick *et al.*, 2003) which showed that "*B. cepacia*" strains could spread between CF patients via simultaneous hospital admissions or social contact outside of the hospital. So, although levels of pulmonary infection caused by Bcc are lower than those seen with pathogens, such as *P. aeruginosa* (Tummler *et al.*, 1999), the emergence of this organism has caused considerable fear and anxiety within the CF community.

Because of the potential for person-to-person spread, significant effort has been expended to gain an understanding of those factors important for transmission. Initially, case-control studies showed that increasing age, underlying severe lung disease, use of aminoglycosides, having a CF sibling colonised with Bcc, and previous hospitalisation were significant risk factors for acquisition (Tablan *et al.*, 1985, 1987). Various routes of initial colonisation have been implicated, but respiratory equipment, particularly

nebulizers, ranks among the most important (Hutchinson *et al.*, 1996). A common feature of published reports of outbreaks of "*B. cepacia*" infections in hospitals is contamination of water supplies, often in pharmacies, disinfectant solutions, tubing for irrigation, nebulized medications and monitoring lines (Martone *et al.*, 1987; Pegues *et al.*, 1996). "*B. cepacia*" has been detected in air samples taken after physiotherapy (Ensor *et al.*, 1996) and can persist in the air after the room is vacated (Humphreys *et al.*, 1994). "*B. cepacia*" can survive for long periods in respiratory droplets on environmental surfaces but this property varies with the strain (Drabick *et al.*, 1996).

In most UK adult CF centres it is now accepted practice to separate patients who are infected with Bcc from those who are not. Guidelines for patients and their carers have been put forward by various CF associations in Europe and North America in an attempt to reduce cross-infection (Chen *et al.*, 2001; Husain *et al.*, 2002). Contemporary advice to patients extends segregation to outside hospitals, directing them not to attend CF meetings, not to have any physical contact with Bcc-negative patients, and to adopt impeccable hygienic behaviour (UK CF Trust Infection Control Group, 1999). It has been documented that such patients have significantly higher rates of anxiety and depression, and significantly lower levels of self-esteem and control than non-colonised patients (Duff *et al.*, 2002).

Transmissible strains

By the early 1990s the development and use of phenotypic and DNA-based genomic typing systems for "*B. cepacia*" made a significant contribution to our understanding of the epidemiology of this organism, including confirmation of epidemic strains (Govan *et*

al., 1985; Kostman *et al.*, 1992; Dasen *et al.*, 1994; Johnson *et al.*, 1994; Cimolai *et al.*, 1995) and confirmation that most chronically colonised patients harbour a single strain of "*B. cepacia*" for prolonged periods (LiPuma *et al.*, 1991). For some years, it has become increasingly evident that the transmissibility of Bcc is strain dependent. The best studied of these transmissible strains is the Edinburgh/Toronto lineage of ET12 (electrophoretic type 12) clone. This is a *B. cenocepacia* strain common among patients in Ontario, Canada and the United Kingdom (Govan *et al.*, 1993; Johnson *et al.*, 1994; Pitt *et al.*, 1996) and characterised by distinctive cable pili (possession of *cblA* gene) and an associated adhesin, which promotes bacterial binding to respiratory epithelia (Sajjan *et al.*, 1995). The intertwined quaternary arrangement of the cable pili does not resemble the arrangement of pili reported for any other bacterial species. This led to the hypothesis that it could reflect an evolutionary selection associated with colonisation of the lung chronically damaged by CF (Sajjan *et al.*, 1995).

Isolates expressing Cable type II pili have been shown to adhere to mucins, and in CF airways, mucins typically form an abnormally thick blanket over the epithelium (Sajjan *et al.*, 1992). Thus the mucus biofilm may provide a favourable niche for colonisation. Binding both to mucins and to the 55 kDa epithelial cell receptor is mediated by the minor pilus component of 22 kDa (Sajjan and Forstner, 1993). The 22 kDa adhesin is not located solely at the appendage tip but is dispersed along the length of the cabled fibre. This distribution would be expected to maximise pilus interactions with mucus networks as well as with receptors on intact or sloughed epithelial cells. By braiding or cabling together, the long individual fibres of the cable pili may be stabilised against rupture by the shearing forces of ciliary beating and chronic coughing. The tendency of the cable pili

to intertangle with similar cables from neighbouring bacteria would be expected to enhance the survival of bacterial colonies (Sajjan *et al.*, 1995). It should be appreciated that the cable pilus (so described for its length and intertwining properties) is not exclusive to this species of Bcc, and neither does the possession of the *cblA* gene alone explain transmission (Mahenthiralingam *et al.*, 1997). The ET12 clone is unusual in containing both the *cblA* gene and a conserved 1.4 Kpb DNA fragment found in other epidemic strains and designated the "*B. cepacia* epidemic strain marker" (BCESM) (Mahenthiralingam *et al.*, 1997). The BCESM region of the genome is unstable, particularly in strains of *B. cenocepacia* III-B, and can be lost after passage *in vitro* (Mahenthiralingam *et al.*, 2002). The BCESM DNA is much more stable in *B. cenocepacia* III-A (Mahenthiralingam *et al.*, 2000a), suggesting that these strains may be the natural hosts for this unusual genomic DNA element. Overall, while it is clear that BCESM DNA is not an absolute marker of the ability to cause infection or spread among CF patients, in CF populations where BCESM-positive strains pre-dominate, they have proved to be highly virulent and very problematic (LiPuma *et al.*, 2001; Mahenthiralingam *et al.*, 2001).

Other epidemic strains, such as the PHDC (Chen *et al.*, 2001) and the "Midwest clone" (Kumar *et al.*, 1997, Coenye *et al.*, 2002), common in the United States, do not express cable pili. All three strains, however, are *B. cenocepacia*. From the studies carried out on CF patient-to-patient spread of Bcc, *B. cenocepacia* would seem to be the main transmissible member of the Bcc (LiPuma, 1999; LiPuma *et al.*, 2001; Mahenthiralingam *et al.*, 2001). Strains residing in other Bcc species and common to multiple patients have been less frequently described (Segonds *et al.*, 1999). However, a recent study by

Biddick *et al.* (2003) found small clusters of patients from a wide geographical area infected with *B. cepacia* and *B. multivorans*, and a large group of patients infected with the same *B. dolosa* strain.

Identification

Despite the advances that have been made in understanding the epidemiology, Bcc infections still have a considerable impact on morbidity and mortality in CF patients (Chaparro *et al.*, 2001; Liou *et al.*, 2001; LiPuma *et al.*, 1998a, 1998b) and therefore, the social and psychological consequences of segregation of Bcc-positive patients places a considerable burden on microbiological laboratories to identify this organism accurately. The taxonomic complexity of *B. cepacia*-like organisms and the lack of widespread and generally accepted identification schemes hinders sound studies that could establish the roles played by, and the pathogenic significance of, the different *B. cepacia*-like organisms. The efficiency of infection control measures are determined by the accuracy with which Bcc is diagnosed, and poor laboratory proficiency in identification of this organism still prevails (Carson *et al.*, 1988; Henry *et al.*, 1997; McMenamin *et al.*, 2000). Although several guidelines intended to enhance accurate identification of bacterial species from sputum culture have been proposed by national CF organisations and by the International *Burkholderia cepacia* Working Group (IBCWG), the degree to which these are followed varies greatly among clinical microbiology laboratories (Shreve *et al.*, 1999).

Phenotypic identification

Phenotypic methods for the identification of Bcc strains and closely related organisms have been difficult to develop. Such methods must be capable of accurately differentiating a diverse variety of Gram-negative, non-fermenting organisms, many of which are closely related in biochemical activity, as well as identifying the individual species comprising the Bcc. In addition these methods should be relatively quick and easy to perform due to the clinical relevance of these organisms.

Several studies have indicated problems with misidentification of Bcc species using phenotypic methods (Kiska *et al.*, 1996; Henry *et al.*, 1997; McMenamin *et al.*, 2000; Shelly *et al.*, 2000; Ferroni *et al.*, 2003). In routine clinical laboratories, the identification of putative Bcc isolates is generally performed using a combination of selective media, conventional biochemical analysis, and/or commercial systems (van Pelt *et al.*, 1999; Shelly *et al.*, 2000). Several different media have been developed for the selective isolation of Bcc isolates from the sputum of CF patients. These media include OFPBL agar, an oxidation-fermentation agar base supplemented with lactose, polymyxin B and bacitracin (Welch *et al.*, 1987); *B. cepacia* medium (PC agar), a commercially available agar containing crystal violet, bile salts, ticarcillin and polymyxin B (Gilligan and Gage, 1985); a medium containing the selective agents 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan and polymyxin B (Wu and Thompson, 1984) and *B. cepacia* selective agar (BCSA), containing lactose and sucrose in an enriched base of casein and yeast extract with polymyxin B, gentamicin and vancomycin (Henry *et al.*, 1997). BCSA was reported to be superior to OFPBL and PC in terms of rapidity and quality of recovery of Bcc

organisms from CF respiratory specimens and inhibition of other organisms (Henry *et al.*, 1999). Organisms not belonging to the Bcc that are capable of growth on BCSA include *B. gladioli* and *Ralstonia spp.* (Henry *et al.*, 1999). The sensitivity and specificity of some or all of the above mentioned media for the isolation of environmental "*B. cepacia*" isolates may be much lower (Carson *et al.*, 1988), and therefore the use of other media like PCAT medium, containing azelaic acid and tryptamine (Burbage *et al.*, 1982) or TB-T medium, containing glucose, asparagine, trypan blue, and tetracycline (Hagedorn *et al.*, 1987) may be recommended.

The abilities of several commercial systems to identify Bcc isolates have been evaluated, and they generally have been proven to have insufficient accuracy (Kiska *et al.*, 1996; van Pelt *et al.*, 1999; McMenamin *et al.*, 2000; Shelly *et al.*, 2000). There are several reports that describe the failure of many commercial test systems to identify Bcc isolates with sufficient sensitivity and specificity. Using commercial systems, members of the Bcc have been misidentified as (among others) *B. gladioli, R. pickettii, Alcaligenes* spp., *Pseudomonas* spp., *Stenotrophomonas maltophilia, Flavobacterium* spp., and *Chryseobacterium* spp., and strains of these various species have likewise been misidentified as belonging to the Bcc (Kiska *et al.*, 1996; McMenamin *et al.*, 2000; Shelly *et al.*, 2000). Commercial test systems with relatively high positive predictive values (including the Vitek GNI Plus and Remel Uni-N/F Tek Plate and N/F Screen; Shelly *et al.*, 2000) are available, but there is nevertheless a general consensus that bacterial isolates presumptively identified as belonging to the Bcc on the basis of commercial test system results should be tested for growth on BCSA, presence of lysine and ornithine decarboxylase activity, oxidation of sucrose and adonitol, presence of

oxidase activity, hemolysis, pigment production, and growth at 42 ^{O}C (Kiska *et al.*, 1996; van Pelt *et al.*, 1999; Shelly *et al.*, 2000; Henry *et al.*, 200). In a recent study, McMenamin *et al.* (2000) found that only one-third of laboratories used biochemical tests to supplement commercial test systems for the identification of "*B. cepacia*". Other phenotypic assays used include serotyping, antimicrobial susceptibility typing, bacteriocin typing and biotyping (Rabkin *et al.*, 1987; 1989).

There may, of course, be phenotypic tests which could potentially identify Bcc and closely related organisms that have not yet been recognised. The differential distribution of enzymes amongst microbial species provides a convenient method for the characterisation and identification of individual taxa for a number of other species, but this area has not been fully investigated for its use in the identification of Bcc strains and many closely related organisms. An enzyme which is released extracellularly is an attractive candidate for detection to aid identification. Such an enzyme may be conveniently assayed using a suitably labelled substrate and a sensitive detector of the cleaved label. For over half a century, diagnostic tests have been employed which are dependent on the possession of particular enzymes, for example, the esculin test (Wasilauskas, 1971, Facklam, 1972). The relative rapidity and ease of applying such tests has led to their wide usage, particularly in kit form, for identification purposes such as the API 20NE (API-bioMérieux, La Balme les Grottes, France), widely used today for the differentiation and identification of non-enteric Gram negative bacilli such as Bcc strains.

Genotypic identification

It is important that diagnostic laboratories employ sensitive methods for detection of Bcc members in order to help guide the management of these patients, as described earlier. To increase the sensitivity and specificity of diagnostic routines, various molecular techniques have been developed. In recent years, phenotypic methods have been largely replaced by these genotypic methods, including macrorestriction digestion of chromosomal DNA followed by pulsed-field gel electrophoresis (PFGE) and various PCR-based fingerprinting techniques (Versalovic *et al.*, 1991; Arbeit, 1995; Olive and Bean, 1999; van Belkum *et al.*, 1996; 2001). Several candidate PCR assays aimed at the identification of "*B. cepacia*" have been described previously (O`Callaghan *et al.*, 1994; Campbell *et al.*, 1995; Karpati and Jonasson, 1996) but most of these assays were developed before the recognition that the Bcc consists of several species. In addition, most relied on published DNA sequence data derived from analyses of culture collection strains that, in retrospect, are poorly representative of the diversity within the Bcc.

Two target genes are commonly used in Bcc analysis: the 16S rRNA gene (O`Callaghan *et al.*, 1994; LiPuma *et al.*, 1999; Segonds *et al.*, 1999) and the *recA* gene (Mahenthiralingam *et al.*, 2000a; McDowell *et al.*, 2001). The *recA* gene polymorphisms enable both differentiation of the Bcc from other closely related bacteria and its differentiation into genomovars. Moreover, the differences in the *recA* sequences within genomovar III led to the establishment of the two *recA* clusters III-A and III-B (Mahenthiralingam *et al.*, 2000a). Species-specific primers have also been developed which can amplify the *recA* gene from most of the Bcc members (Mahenthiralingam *et*

al., 2000a). Phylogenetic analysis of *recA* from a range of bacteria has demonstrated that the gene may be very useful for the separation of closely related species and may define evolutionary trees that are consistent with those observed for rRNA genes (Eisen *et al.*, 1995; Karlin *et al.*, 1995; Mahenthiralingam *et al.*, 2000a). A nested PCR assay has also been developed that detects the *recA* gene of the Bcc in sputum (Drvinek *et al.*, 2002). The product of the first PCR round is also used to identify the genomovar of the pathogen.

As *B. anthina* and *B. pyrrocinia* have been formally identified as members of the Bcc only very recently (Vandamme *et al.*, 2002), few studies have been carried out on their genotypic identification. However, the *recA* RFLP patterns of the *B. anthina* isolates differed from those of the recognised members of the Bcc and from that of the *B. pyrrocinia* type strain (Vandamme *et al.*, 2002).

Typing

One of the first methods reported for typing "*B. cepacia*" isolates was ribotyping (LiPuma *et al.*, 1990). The most widely used typing methods are now PFGE and random amplified polymorphic DNA analysis (Mahenthiralingam *et al.*, 2001). Multilocus restriction typing is another method for genotyping Bcc isolates, in which variation at several loci is indexed by restriction analysis of PCR-amplified genes (Coenye and LiPuma, 2002). Among these techniques, PFGE is generally considered as the "gold standard" in bacteriological typing (Arbeit *et al.*, 1995; Tenover *et al.*, 1995; Olive *et al.*, 1999), and a number of studies have applied PFGE to assess Bcc epidemiology (Segonds

et al., 1997; Vandamme *et al.*, 2000; Agodi *et al.*, 2001; Chen *et al.*, 2001; Coenye *et al.*, 2002).

Treatment of Bcc infections

Antibiotic resistance

The development of resistance to antibiotics occurs frequently in CF infections due to the intensive selective pressure provided by the large numbers of antibiotics used in these patients (Doring *et al.*, 2000). It seems reasonable to speculate that the characteristic multi-drug resistance of Bcc isolates to antimicrobial agents might have played a role in its increased prevalence during the 1980s. A major aspect of CF patient care has centred on antibiotic therapy to ameliorate the effects of acute pulmonary infections/exacerbations in these patients. Previous studies have provided evidence that although eradication of organisms from the lower respiratory tract is not possible in patients with CF, decreased bacterial density and decreased bacterial virulence factor production after antibiotic therapy leads to a decrease in inflammation and clinical improvement in these patients (Smith *et al.*, 1999; Geers *et al.*, 1987; Grimwood *et al.*, 1989a; Cantin *et al.*, 1993).

Intrinsic resistance of Bcc

Intrinsic resistance to antibiotics is generally high in Bcc strains. Most strains are resistant to most, if not all, ß-lactams as well as to polymyxin, ciprofloxacin, imipenem

and also the aminoglycosides, one of the major classes of drugs used to treat CF lung infections, including gentamicin and tobramycin (Pitt *et al.*, 1996). The rate of resistance to the ß-lactam agents is generally high, with the exception of meropenem, which appears to have improved *in vitro* activity.

ß-lactam resistance

The resistance of "*B. cepacia*" to ß-lactams is most strikingly seen in its ability to utilise penicillin G as a sole carbon source (Beckman *et al.*, 1979), and has been associated with a number of mechanisms including the presence of ß-lactamase enzymes, small porin channels and (for high level resistance) decreased porin expression. Several ß-lactamase enzymes have been described for "*B. cepacia*" (Trepanier *et al.*, 1997). Expression of *penA* ß-lactamase is induced by low levels (150 µg per ml) of penicillin (Joris *et al.*, 1994). This enzyme is not inhibited by clavulanic acid or sulbactam and is highly active against piperacillin and azlocillin (Prince *et al.*, 1988).

Outer membrane permeability

In the past the intrinsic resistance of Gram negative bacteria has often been attributed largely to the presence of the outer membrane barrier. The narrow porin channels in the outer membrane are thought to slow down the penetration of antibiotics into the cell. This is particularly true for "*B. cepacia*" where the porin channel is unusually small, as indicated by its small single channel conductance (Parr *et al.*, 1987). This porin channel was shown to be approximately 10 times less permeable than the equivalent channel in *E.*

coli, and was equal to that found in *P. aeruginosa* (Parr *et al.*, 1987). It has also been shown that there are a low number of phosphate and carboxylate groups in the lipopolysaccharide (LPS) of the "*B. cepacia*" outer membrane. This may contribute to its resistance to cationic antibiotics, such as polymyxin and the aminoglycosides, due to ineffective binding of the antibiotic to the outer membrane (Cox *et al.*, 1991).

Multi-Drug resistance pumps - efflux

It has been shown that a variety of bacteria possess pumps of low specificity that can exclude structurally unrelated toxins, including antibiotics such as chloramphenicol, quinolones and trimethoprim (Burns *et al.*, 1989). These pumps are known as multi-drug resistance (MDR) pumps and their presence in pathogens like the Bcc can pose a serious threat to effective treatment. Such pumps have been reported in strains of *P. aeruginosa* (Narita *et al.*, 2003; Sekiya *et al.*, 2003; He *et al.*, 2004) and "*B. cepacia*" (Wigfield *et al.*, 2002).

Resistance plasmids have been described in "*B. cepacia*" but their contribution to overall resistance is unknown (Sabate *et al.*, 1994). Conjugative transfer from *P. aeruginosa* to "*B. cepacia*" and from "*B. cepacia*" transconjugants to other strains of the same species has been demonstrated (Lennon and DeCicco, 1991).

Antibiotic treatment

A study of "*B. cepacia*" isolates from CF patients in Britain found that three quarters of isolates were susceptible to ceftazidime, piperacillin (plus tazobactam) and meropenem but usually at only high breakpoint levels (Pitt *et al.,* 1996). Additional agents with some *in vitro* activity include trimethoprim and chloramphenicol. Various combinations of 2, 3 or even 4 antibiotics may exhibit synergy *in vitro* against "*B. cepacia*" (Kerr *et al.*, 1993; Burns *et al.*, 2000). Recent randomised, placebo-controlled trials have shown that combination antibiotic therapy with two antipseudomonal antibiotics is superior to antibiotic monotherapy, and results in a significant reduction in bacterial colony counts and a more prolonged clinical remission in CF patients who are infected with *P. aeruginosa* (Regelmann *et al.*, 1990, Smith *et al.*, 1999). Unfortunately, many CF associated *P. aeruginosa* and Bcc isolates are now multiresistant to single antibiotics. Traditional microbiology laboratory susceptibility results for single antibiotics are often inadequate for these multiresistant bacteria, with the result that the choice of a combination antibiotic regimen for CF patients infected with these multiresistant organisms can often only be made empirically. The danger with this approach is that empirically chosen antibiotic combinations may not be bactericidal against multiresistant *P. aeruginosa* and may result in a less than optimal treatment outcome.

Virulence and pathogenesis of Bcc and *P. aeruginosa* infections in CF

There is a wide variation in disease severity among CF patients infected with the Bcc. Some strains seem to be associated with a more severe illness than others (Aris *et al.*,

2001; Mahenthiralingam *et al.*, 2001). These observations have raised the possibility that only some strains have specific features that predispose them to human disease. Knowledge about these virulence factors and pathogenesis of the Bcc is scanty, although the persistent and sometimes invasive infections caused by the Bcc suggest that the organisms possess mechanisms for both cellular invasion and evasion of the host immune response (Martin *et al.*, 2000). "*B. cepacia*" however, appears to produce few recognised virulence factors, and animal models of infection indicate that it is less virulent than *P. aeruginosa* (Stover *et al.*, 1983). Some strains of "*B. cepacia*" do produce a range of virulence factors including exopolysaccharide, lipopolysaccharide, ornithine amine lipids, proteases, lipases, haemolysin, siderophores, phospholipase C, melanin pigment and antibiotic resistance systems (Wilkinson and Pitt *et al.*, 1995; Cerantola *et al.*, 1996; Speert 2002), although evidence that any of these factors play a major role in pathogenesis is limited. Nor is there any convincing evidence the Bcc produces any of the major virulence factors seen with *P. aeruginosa*, such as exotoxin A, cytotoxins or alginate (Nelson *et al.*, 1994). Expression of cable pili is the only genetically characterised virulence factor so far described.

Cable pili

Cable pili bind specifically to CF respiratory mucin and airway epithelial cells. The presence of the *cblA* gene in strains is associated almost uniquely with transmissibility (Sun *et al.*, 1995); a novel insertion sequence IS1356 is also found in the majority of CblA+ strains (Tyler *et al.*, 1996). Electron microscopy studies have shown that approximately 60 % of "*B. cepacia*" strains express peritrichous pili (Kuehn *et al.*,

1992), and that some strains possess polar pili (Saiman *et al.*, 1989). It has also been shown that "*B. cepacia*" pilin peptides are more similar to *E. coli* pilins than to those of *P. aeruginosa* (Sajjan *et al.*, 1995). The pili for both "*B. cepacia*" and *P. aeruginosa* bind to the same residues present in many asialoglycolipids (Saiman *et al.*, 1990). Despite this, however, there does not seem to be competition for the same epithelial receptors, indicating that different epithelial receptors may be used preferentially by each of the bacterial species or that the bacteria may bind to each other. Indeed binding of two strains of "*B. cepacia*" to epithelial monolayers increased in the presence of *P. aeruginosa,* indicating a possible synergistic relationship between the two species (Saiman *et al.*, 1990). Not all patients however are colonised with both bacteria; the Edinburgh CF clinic reported that 38 % of patients colonised with "*B. cepacia*" were not co-colonised with *P. aeruginosa* (Govan *et al.*, 1993).

Inflammation and invasion

The inflammatory response in CF is not well understood. It is not known for certain whether the inflammation seen is initiated by the CFTR defect or by infections with various pathogens. It has been found that "*B. cepacia*" secretory products are potent pro-inflammatory agents for respiratory epithelium and suggests functional CFTR is not required for cytokine or prostanoid responses (Fink *et al.*, 2003). The increased morbidity and mortality observed with "*B. cepacia*" infection may therefore be explained, in part, by this inherently pro-inflammatory character of the organism (Corey *et al.*, 1996; Frangolias *et al.*, 1999). "*B. cepacia*" derived LPS elicits a 9-fold higher release of cytokines from leucocytes compared to *P. aeruginosa* (Shaw *et al.*, 1995).

Therefore, the Bcc may significantly contribute to the vicious cycle of infection and inflammation within the CF lung.

It has been shown that IgG antibodies to *P. aeruginosa* outer membrane proteins (OMP) cross-react with those of "*B. cepacia*" (Lacy *et al.*, 1995). However, previous colonization with *P. aeruginosa* and the ensuing antibody response does not seem to prevent subsequent colonization by "*B. cepacia*" and thus OMP antibodies to "*B. cepacia*" are not protective (Aronoff *et al.*, 1991). Anti-core LPS antibodies have also been shown to be specific for "*B. cepacia*" (Nelson *et al.*, 1993; Lacy *et al.*, 1995). Again these antibodies do not prevent bacterial colonization, but their detection in serum can provide useful confirmation of Bcc colonisation.

Kenna *et al.* (2003) found that neither O-serotype nor phage susceptibility correlate with genomovar status. They concluded that variability in LPS may contribute to the success of these highly adaptable bacteria as human pathogens. Antibodies which appear to confer some protection against infection have been identified, and the presence of IgA antibody against a 30 kDa porin has been associated with better prognosis, and it has been suggested that this antibody may potentially be exploited for immunotherapy (Burnie *et al.*, 1995). Other studies have shown that all strains expressing rough lipopolysaccharide (R-LPS) are sensitive to the bactericidal effect of human serum. This is surprising, as many strains of "*B. cepacia*" isolated from CF patients, including the epidemic strain, are known to possess the R-LPS phenotype. This evidence gives strong support to the hypothesis that "*B. cepacia*" isolates may invade and survive within respiratory epithelial cells, enabling the organism to persist within the CF host (Burns *et*

al., 1992). "*B. cepacia*" can be found within host cells of infected mice (Chiu *et al.*, 2001).

A study by Keig *et al.* (2002) investigated whether there are differences between members of the Bcc in their ability to invade human respiratory epithelial cells and they found that *B. multivorans and B. cenocepacia* strains were more invasive than those of *B. cepacia, B. stabilis* and *B. vietnamiensis*. It has been suggested that the distinct invasion pathways employed by the Bcc may account for differences in virulence between Bcc members (Schwab *et al.*, 2002). "*B. cepacia*" can also survive within macrophages and in amoebae (Marolda *et al.*, 1999). The latter cell is particularly interesting since it could provide insights into the reservoir within which Bcc isolates survive in the environment. If the Bcc does indeed have the capacity for intracellular parasitism, this raises important issues with regard to therapy; the optimal drug would be one that is able to penetrate eukaryotic cells. No correlation between invasiveness and other putative virulence factors of importance in Bcc infection in individuals with CF, i.e. cable pilus and the BCESM marker, was identified. The mechanisms that permit the Bcc to cause bacteraemia are not yet known but probably involve sequential penetration of airway barriers (Keig *et al.*, 2002).

There is evidence of intercellular communication between *P. aeruginosa* and "*B. cepacia*"; addition of *P. aeruginosa* PAO1 exoproduct appears to significantly increase the production of protease, lipase and siderophore in "*B. cepacia*". This interaction may play an important role in the pathology of the CF lung in patients who are co-colonised

with both of these organisms, and is one of the first examples of interspecies communication recorded (McKenny *et al.*, 1995).

Biofilms

Many bacterial infections evolve in the form of communities of bacteria (biofilms) in which there is a type of communication among the individual bacteria when they sense a minimum cell density, or "quorum" (Donlan, 2001). "Quorum sensing" is associated with enhanced resistance to antimicrobial agents (Stewart and Costerton, 2001) and with the elaboration of quorum sensing molecules, known as autoinducers, (Riedel *et al.*, 2001). These autoinducers, which are acylated homoserine lactones (AHLs), have substantial and multiple effects on the expression of various genes. Several of the genes in *P. aeruginosa*, the expression of which is controlled in part by autoinducers, are considered to be virulence determinants. Thus bacterial density is probably very important in dictating the level of toxin production as well as antibiotic resistance during chronic *P. aeruginosa* infection in CF. Bacteria from all genomovars of the Bcc encode the genes for elaboration of AHLs (Lewenza *et al.*, 1999). Conway *et al.* (2002) also demonstrated that all of these species elaborate AHLs but to greatly different degrees. Many of the strains also form biofilms but there is no correlation between the extent of biofilm formation and the amount of AHL production. Since Bcc isolates cause chronic infection in some patients with CF and since biofilms are such an important feature of chronic infections, the role of quorum sensing in the pathogenesis of Bcc infection deserves further investigation.

Environmental aspects of Bcc

"*B. cepacia*" can be isolated from surface waters and soil, but with varying frequency (Butler *et al.,* 1995). It is almost exclusively phytopathogenic, through the production of pectolytic enzymes. "*B. cepacia*" rhizosphere isolates grow over a wider range of temperatures and differ from CF isolates in a number of other properties (Bevivino *et al.*, 1994). It is relatively infrequent in salads and fresh foods (Fisher *et al.*, 1993) but it was recovered from 18 % of domestic environments by Mortensen *et al.* (1995). An epidemiological study of "*B. cepacia*" acquisition during four CF summer camps revealed that four environmental isolates (3 cultured from lake water, and 1 cultured from iced drinking water out of 58 environmental samples) had ribotypes distinct from any of the "*B. cepacia*" strains present in, or acquired by campers (Honicky *et al.*, 1993). Analysis of accumulated data has led to the speculation that the principal source for "*B. cepacia*" acquisition by CF patients is the respiratory secretions of colonised patients, rather than environmental isolates (Pitt and Govan, 1993).

Commercial use of Bcc

Bcc organisms may have commercially useful properties and have been used in agriculture as biocontrol agents and in the bioremediation of toxic agents (LiPuma and Mahenthiralingam, 1999). Several "*B. cepacia*" strains have attracted attention as antagonists of soil-borne plant pathogens and as plant growth-promoting agents since they can colonize the rhizosphere of several economic crops (Holmes *et al.*, 1998; LiPuma and Mahenthiralingam, 1999). Such strains thereby increase crop yield and, as a

result, have attracted interest as biological control agents (Parke *et al.*, 1991; McLoughlin *et al.*, 1992). The exceptional metabolic diversity of this organism, which allows it to use, e.g., constituents of crude oils and herbicides as carbon sources, could be put to use in the bioremediation of recalcitrant xenobiotics (Coenye *et al.*, 2001a). However, most strains used or under development for biocontrol or bioremediation purposes are taxonomically poorly characterised, and their potential hazard to the CF community is unclear (Govan *et al.*, 2000).

Aims

The four principal aims of the study were:

1 - To set up and optimise the *recA* RFLP analysis technique using a panel of genetically defined experimental strains (Mahenthiralingam *et al.*, 2000b), and to determine the genomovar status of a collection of clinical Bcc isolates cultured from CF patients (identified previously by basic phenotypic tests), followed up by clonality analysis of these isolates.

2 - To screen a large number of fluorogenic and chromogenic enzyme substrates against genetically defined Bcc strains and closely related organisms to evaluate their potential as markers for specific identification of the Bcc and/or the individual species of the Bcc.

3 - To evaluate the potential of the oxidation of various carbohydrates as candidates for differentiation between the species of the Bcc.

4 - To evaluate the antimicrobial compound alafosfalin in combination with a number of cell wall-acting antibiotics, against 19 clinical isolates of *P. aeruginosa* cultured from CF patients and 19 genetically defined Bcc strains.

CHAPTER 2

Genotypic identification and clonality study on Bcc strains isolated from patients attending the Freeman Hospital Cardiopulmonary Transplantation Unit

Introduction

Before the taxonomic complexity of the Bcc was recognized, molecular investigation involving *P. cepacia* (reclassified as *Burkholderia cepacia* in 1992) mainly centered on the epidemiological aspects of this organism (LiPuma *et al.,* 1988; Stull *et al.,* 1988; Anderson *et al.,* 1991; Kostman *et al.,* 1992; Bingen *et al.,* 1993; Dasen *et al.,* 1994; Johnson *et al.,* 1994). However, since the complex taxonomy of this organism has emerged, much emphasis has been placed on determining molecular techniques capable of differentiation between the growing number of species comprising the Bcc (Vandamme *et al.,* 1997; Bauernfeind *et al.,* 1999; LiPuma *et al.,* 1999), as well as more specific epidemiological studies focusing on national and global transmission of the individual Bcc species (Coenye *et al.*, 2002; Heath *et al.*, 2002; Coenye and LiPuma, 2003).

Epidemiological investigation of "*B. cepacia*"

Spread of respiratory infections in patients with CF had not been recognized as a significant clinical problem up until the early 1990s. However, molecular epidemiological studies were performed previous to this when *P. cepacia*, as it was known then, was being recovered from hospital environmental surfaces but nosocomial transmission had not been documented (Isles *et al.*, 1984; Tablan *et al.*, 1985; Hardy *et al.*, 1986; LiPuma *et al.*, 1988). LiPuma *et al.* (1988) developed a typing system that allowed differentiation of these *P. cepacia* isolates on the basis of analysis of chromosomal restriction fragment length polymorphisms detected by hybridization with ribosomal ribonucleic acid.

However, it soon became apparent in the early to mid 1990s that *P. cepacia* was capable of nosocomial transmission due to the employment of a number of techniques for typing such as traditional ribotyping, PCR ribotyping, random amplified polymorphic DNA (RAPD) fingerprinting, multilocus enzyme electrophoresis and pulsed-field gel electrophoresis (PFGE) (LiPuma *et al.*, 1988; LiPuma *et al.*, 1990; Anderson *et al.*, 1991; Kostman *et al.*, 1992; Bingen *et al.*, 1993; Govan *et al.*, 1993; Smith *et al.*, 1993; Johnson *et al.*, 1994; Sun *et al.*, 1995; Mahenthiralingam *et al.*, 1996; Segonds *et al.*, 1997). Epidemiological observations suggested that transmission had been due to direct patient-to-patient contact both within and outside the hospital setting, (Mahenthiralingam *et al.*, 1996). In addition to risk of spread, infection was also linked to a rapid decline in clinical condition in certain CF patients, which became known as "cepacia syndrome", as described earlier.

Identification of Bcc species

As it became apparent that "*B. cepacia*" was actually a group of at least five distinct genomic species, molecular techniques became essential tools not only in the continued epidemiological study of these different genomovars, but also in identification (Vandamme *et al.*, 1997). The term genomovar was introduced around 1997 to denote phenotypically similar but genotypically distinct groups of strains (Vandamme *et al.*., 1997). These genomovars share a low level of DNA hybridization and therefore represent distinct species for which official bionames were assigned as differential diagnostic tests became available. There are currently nine species in the Bcc, all of which have designated species names.

Infection with Bcc strains can have devastating consequences for CF patients, including development of "cepacia syndrome", rejection as potential lung transplant recipients at many CF centres due to potentially poor outcomes, in particular with *B. cenocepacia* infections, as well as colonised patients being excluded from social events and scientific conferences for CF patients (Aris *et al.*, 2001; Chaparro *et al.*, 2001; De Soyza *et al.*, 2001, 2003). Unfortunately, the diversity of Bcc organisms has made precise isolation and identification difficult. As phenotypic variation makes identification of the different species very difficult, definitive identification of the Bcc genomovars relies on molecular analysis (Bauernfiend *et al.*, 1999; Coenye *et al.*, 1999; van Pelt *et al.*, 1999; Mahenthiralingam *et al.*, 2000, 2002).

Although the Bcc species could be discriminated by techniques such as DNA-DNA and rRNA hybridisation, whole cell protein analysis and fatty acid profiles, these procedures are not suitable for routine diagnostic work as they require high technical skill and are time consuming. The most commonly adopted molecular techniques for Bcc species identification are PCR-based techniques (Mahenthiralingam *et al.*, 2000a; McDowell *et al.*, 2001; Moore *et al.*, 2002; Vermis *et al.*, 2002). Molecular diagnostic probes based on PCR provide a rapid and frequently highly discriminatory means of microbial identification.

16S/23S PCR analysis

A number of the studies regarding PCR-based identification of members of the Bcc have been based on the diversity within the nucleotide sequences of the 16S and/or23S rDNAs and were either aimed at the development of species-specific primers or RFLP

analysis of the PCR-amplified 16S rRNA gene (Bauernfeind *et al.*, 1999; LiPuma *et al.*, 1999a; Segonds *et al.*, 1999; Brisse *et al.*, 2000; Mahenthiralingam *et al.*, 2000a; Whitby *et al.*, 2000a, 2000b; Coenye *et al.*, 2001a, 2001b). The results from these studies clearly indicated that the procedures facilitated rapid and specific identification of *B. multivorans, B. vietnamiensis, B. dolosa* and *B. gladioli*, and each could be separated from all other members of the Bcc. Although *B. cepacia, B. cenocepacia, B. stabilis, B. ambifaria* and *B. pyrrocinia* could be identified as a group, the variation within the rRNA operon is too small to separate all members of the Bcc. Segonds *et al.* (1999) found that using the restriction enzymes *Alu*I, *Cfo*I, *Dde*I and *Msp*I on the amplification product of the 16S rRNA gene, it was possible to discriminate between *B. cepacia* and *B. cenocepacia.* It was generally agreed that although widely used for bacterial systematics, recent results demonstrated that analysis of 16S rDNA was limited in its ability to differentiate the species of the Bcc. Nucleotide sequence variation within this gene is not sufficient to enable all current genomovars within the complex to be easily identified.

recA gene PCR analysis

Due to the discriminatory limitation of 16S/23S PCR, Mahenthiralingam *et al.* (2000a) developed a novel PCR-based identification assay based on analysis of the *recA* gene. They discovered that there was sufficient variation in nucleotide sequence of the *recA* gene to enable discrimination of the first five genomovars described at that time (now *B. cepacia, B. multivorans, B. cenocepacia, B. stabilis* and *B. vietnamiensis*). The *recA* gene shows 94 % to 95 % similarity between the different genomovars, and typically

98 % to 99 % similarity can be found within the genomovars. However, *B. cenocepacia* contains two subpopulations with a different recA allele (III-A and III-B).

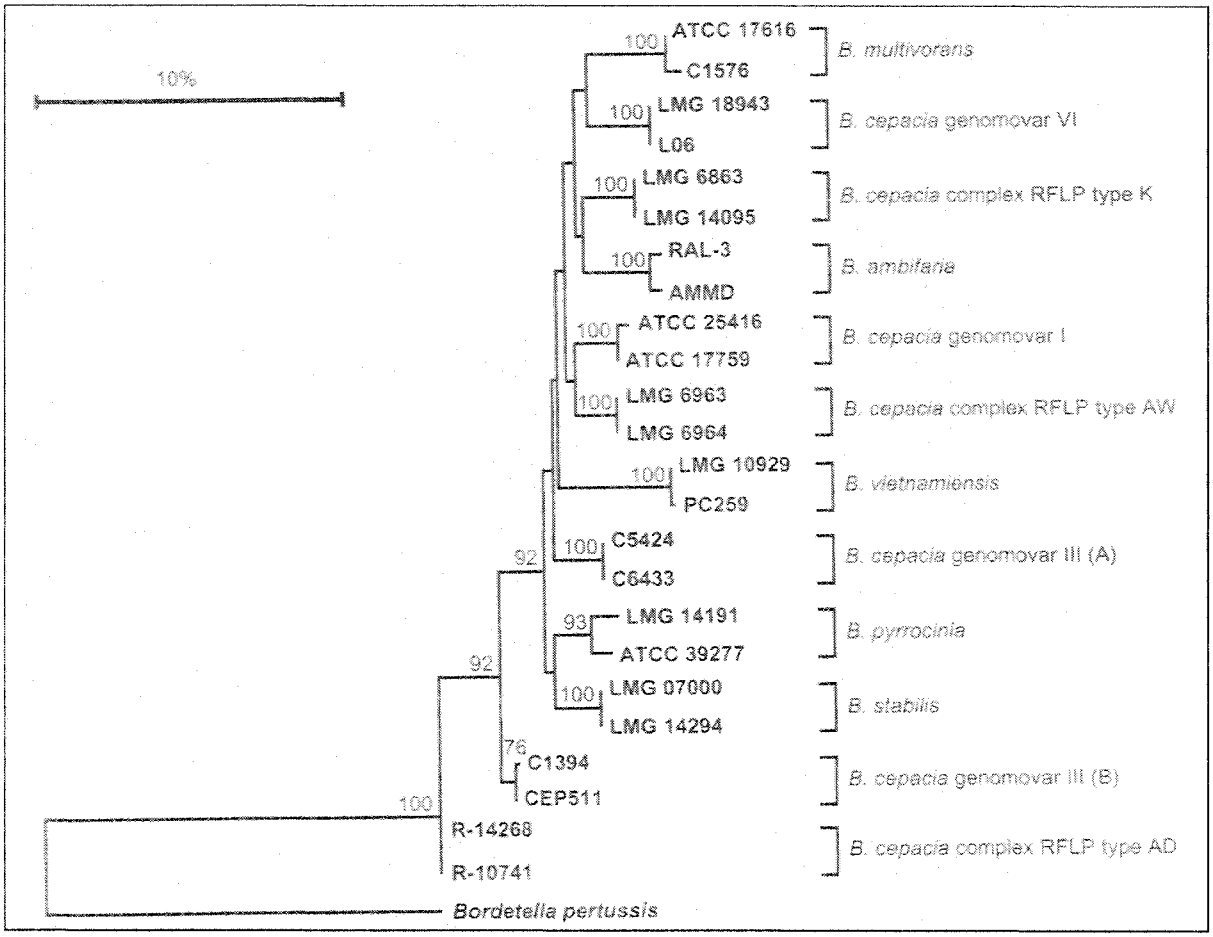
For PCR of this gene, primers BCR1 and BCR2 were designed from homologous sequences at the 5' and 3' ends of the *recA* open reading frame. These primers amplified a single 1 Kb amplicon from all strains representative of the five then members of the Bcc. Amplification of the *recA* gene with primers BCR1 and BCR2 was used as an initial means of placing an isolate within the Bcc since cross reaction of these primers with other species commonly found in CF sputum was not detected, including *P. aeruginosa, B. gladioli, R. pickettii* and *Pandoraea* sp. RFLP types generated by digestion with the enzyme *Hae*III were the most discriminatory among the four enzymes tested. *Hae*III RFLP analysis was capable of discriminating among all five genomovars except for one RFLP pattern which was shared by one of the *B. cenocepacia* strains, and all of the *B. stabilis* strains examined. To distinguish *B. cenocepacia* and *B. stabilis* with this RFLP type, digestion with an additional enzyme (such as *Mnl*I) was required.

Mahenthiralingam *et al.* (2002) subsequently found that analysis of *recA* sequence variation, in general, correlated well with the genomovar taxonomy. RFLP analysis of the *recA* gene can serve as a primary means of identifying taxonomic diversity among isolates and >50 Bcc RFLP types have now been found when the gene is cut with the restriction enzyme *Hae*III. Novel *recA* gene RFLP types that do not correlate with a known genomovar can then be subjected to nucleotide sequence analysis to enable phylogenetic predication of genomovar status. Phylogenetic analysis of *recA* gene sequences from strains representative of all nine current Bcc genomovars is shown in

Figure 2.1. All nine Bcc genomovars separate into distinct arms of the phylogenetic tree. The diagram also indicates the positions of strains of novel *recA* RFLP types AD, AW and K. This specificity has led to the successful application of *recA* PCR directly to CF sputum (McDowell *et al.*, 2001). Such direct testing for suspected Bcc infection may prove very useful in hospital infection control and the clinical management of CF patients.

RecA species-specific primer pairs have also been designed for the identification of *B. cepacia, B. cenocepacia, B. multivorans, B. stabilis, B. vietnamiensis* and *B. ambifaria* (Mahenthiralingam *et al.*, 2000a), enabling the recognition of multiple types within each genomovar. However, these genomovar-specific *recA*-based PCR tests appear to be less specific in light of the identification of further taxonomic diversity. For example, the PCR primers designed to be specific for *B. cepacia* genomovar I fail to detect some genomovar I isolates and cross-react with *B. pyrrocinia* (Mahenthiralingam *et al.*, 2002). Nucleotide sequence determination of *recA* provides a powerful means of both identification and classification of these poorly defined bacteria, although not suitable for routine laboratory diagnosis (Miller and Mahenthiralingam, 2003).

Figure 2.1: Phylogenetic tree of *recA* sequences from strains of the current Bcc species.



Vermis *et al.*, (2002)

Pulsed-Field Gel Electrophoresis (PFGE)

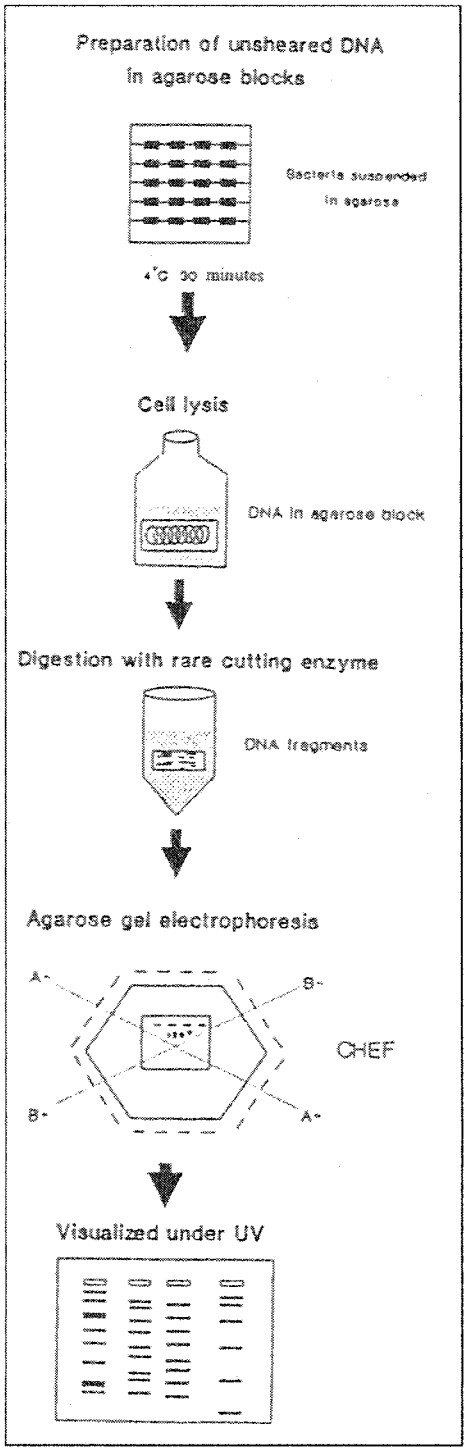
Current epidemiological studies take into consideration the taxonomic complexity of the Bcc. Although such studies account for the different species comprising the Bcc, the same techniques used before the more recent taxonomic revelations were apparent are still adopted. These include RAPD profiling (Clode *et al.*, 2000), ribotyping (Brisse *et*

al., 2000), PFGE (Heath *et al.*, 2002), and multilocus restriction typing (Coenye and LiPuma 2002).

A great leap forward was made in 1982 when Schwartz *et al.* introduced the first pulsed field gel electrophoresis method. This and the large number of related variations on it developed since have increased the size limit for nucleic acid separations by two orders of magnitude or more to at least 12 Mb (Orbach *et al.*, 1988). Such techniques have various application, for example they have made possible the separation of intact yeast chromosomal DNAs (Carle and Olsen, 1984; Schwartz and Cantor, 1984), and the mapping of the Duchenne muscular dystrophy gene (Kenwick *et al.*, 1987), of the genome of *E. coli* (Smith *et al.*, 1987) and human chromosome 21 (Gardiner and Patterson, 1989).

Classification of genomic DNA restriction patterns by PFGE has been shown to be a useful tool for investigating the source, transmission, and spread of nosocomial infections particularly for epidemiologic typing of Bcc strains, for example in studying ET-12 strains (Tenover *et al.*, 1995). The PFGE procedure used for typing such bacteria is illustrated in Figure 2.2.

Figure 2.2: A schematic representation of the pulsed-field gel electrophoresis technique for typing bacteria



Al-Thani, (1998)

Experimental objectives

1 - To gain an understanding of the nature and emerging pattern of Bcc infections amongst CF patients attending the Freeman Hospital Cardiopulmonary Transplant Unit (serving CF units in Northern England, Scotland and both Northern and Southern Ireland), including those referred for pre-transplant assessment, by conducting a molecular study to determine the genomovar status of isolates recovered since 1989 using *recA* PCR followed by RFLP analysis with restriction enzymes *Hae*III and *Mnl*I.

2 - To determine whether patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit were harboring unique or transmissible strains (in particular the ET-12 clone) using PFGE analysis, and also whether there was a change in Bcc strain between pre- and post-transplant colonisation.

Materials

Bacterial strains

Control organisms

Thirty organisms were obtained from the Belgian Coordinated Collections of Microorganisms (BCCM), which comprised a documented reference panel of genetically defined Bcc strains of genomovars I - V (Mahenthiralingam *et al.*, 2000b).

Clinical isolates

Expectorated sputum was routinely collected from patients during pre-transplant assessment and, when necessary, immediately before surgery and post-transplant. Intra-operative bronchoalveolar lavage (BAL) and post-transplant BAL (at day 7) were also collected from patients undergoing surgery. Presumptive Bcc bacteria were isolated by culture on selective agar (Columbia blood agar with 5 % defibrinated horse blood and colomycin, vancomycin supplement). Phenotypic analyses were performed using the API 20NE diagnostic test (bioMérieux, Marcy l'Etoile, France), following manufacturers instructions. Antibiotic sensitivities were identified by disc diffusion tests. A total of 112 pre- and post-transplant isolates, recovered from 44 patients referred from nine different CF centres, were archived from 1989 to 2002 as pure cultures on lenticules as previously described (Codd *et al.*, 1998; Lightfoot *et al.*, 2001) at -20 ^{O}C pending molecular analysis (see Appendix 2.1).

Growth media

Defibrinated horse blood was obtained from TCS Biosciences Ltd. (Buckingham, UK). Columbia agar and brain heart infusion (BHI) broth were obtained from Oxoid (Basingstoke, UK).

DNA extraction, amplification and restriction enzyme digestion

DNA extraction from all organisms was achieved using the High Pure PCR Template Preparation Kit from Roche Diagnostics (Welwyn Garden City, UK). The lysozyme used in DNA extraction, gel loading solution (0.05 % w/v bromophenol blue, 40 % w/v sucrose, 0.1 M EDTA pH 8.0), 100 bp ladder (containing 10 bands ranging from 100 to 1000 bp in exact 100 bp increments) and Lambda *Hind*III ladder (containing 8 bands, sizes 125, 564, 2027, 2322, 4361, 6557, 9416, 23130 bp) were obtained from Sigma-Aldrich Company Ltd. (Poole, UK). BCR1/2 primers were obtained from MWG Biotech AG, (Milton Keynes, UK). Thermophilic DNA polymerase 10 x buffer (magnesium free), magnesium chloride solution, deoxyribonucleotide triphosphates (dNTPs) and *Taq* polymerase, used in the PCR reactions, and Bovine Serum Albumin, *Hae*III and *Mnl*I restriction enzymes and respective reaction buffers used for restriction enzyme digestion, were all obtained from Promega (Southampton, UK). High-Pure Low EEO Agarose used for DNA band separation was obtained from Biogene (Kimbolton, UK).

Other chemicals and solvents

Ethidium bromide was obtained from BDH (Poole, UK). Phosphate buffered saline (PBS), dimethyl sulphoxide (DMSO) and ethanol were obtained from Sigma-Aldrich Company Ltd. (Poole, UK). TAE buffer (10 x strength) was obtained from Invitrogen™ (Paisley, UK).

Equipment

Small volumes were dispensed using calibrated Gilson semi-automatic pipettes (P20, P200 and P1000) with sterile disposable tips (Gilson Medical Electronics, Villiers-le-Bel, France). Large volumes were dispensed using sterile disposable 10 ml pipettes (L.I.P. Ltd, Shipley, UK). Plastic consumables, including 1 µl loops, 3 ml plastic graduated pastettes, 25 ml universals and sterile Petri dishes, were obtained from Bibby Sterilin Ltd. (Aberbargoed, UK). All 1.5 ml and 0.2 ml microcentrifuge tubes were obtained from Roche Diagnostics (Welwyn Garden City, UK). The pH of the phosphate buffered saline used for DNA extraction was measured using a pH meter (Hanna Instruments Ltd, Leighton Buzzard, UK). The centrifugation stages of the DNA extraction were performed using a Micromax micro centrifuge (IEC, London, UK). Desired amounts of agarose were weighed out using a Satorius 2434 electronic balance; accurate to 0.1mg (Satorius Ltd, Epsom, UK) and the agarose gels prepared in a 13 cm by 15 cm gel tray (Anachem, Bedfordshire, UK). Electrophoresis was performed in a gel tank (Anachem, Bedfordshire, UK). The power source for the electrophoresis was a Chamber Master Plus, (Helena Laboratories Ltd, Gateshead, UK). DNA concentrations

were elucidated using Quartz cuvettes (Hellma UK Ltd, Southend on Sea, UK) and a Helios alpha spectrophotometer (Spectronic Unicam, Leeds, UK).

The amplification of the *recA* gene was performed using a Perkin Elmer 9600 thermocycler (from Perkin Elmer, Buckinghamshire, UK). The extracted genomic DNA, the amplified *recA* gene and the restriction fragment length polymorphisms were visualised using the Gel Doc system (Biorad, Hemel Hempstead, UK), and the resulting photographs printed on thermal paper (Biogene, Kimbolton, UK).

Methods

Bacterial strain preparation

The control BCCM strains were reconstituted by adding 1 ml of BHI and subsequently spreading the suspension onto blood agar. Plates were incubated for 48 - 72 hrs at 30 ^{O}C. These strains were then stored as pure cultures on lenticules as described for the clinical isolates.

Culturing lenticules

To culture a strain or isolate, a lenticule was removed from storage, placed on the surface of a 5 % horse blood Columbia agar plate and left for 10 mins to rehydrate. The resulting drop was spread for single colonies using a sterile 1 µl loop and the plate was then incubated for 18 hr at 37 °C in aerobic conditions.

DNA extraction

Once cultured from lenticule, single colonies of each strain or isolate were inoculated into brain heart infusion broth (BHI) and incubated overnight at 37 ^{O}C. An aliquot of 500 µl of each suspension was transferred to a 1.5 ml microcentrifuge tube and the sample collected by low speed centrifugation (1000 g) for 5 mins. Bacterial pellets were resuspended in 200 µl PBS and genomic DNA was extracted from all strains using the High Pure PCR template preparation kit according to manufacturers instructions.

Preparation of agarose gels

A 13 cm by 15 cm gel consisting of 80 ml of 1 % agarose was required. This was prepared as follows:

Agarose (0.8 g) was placed in a clean 250 ml conical flask, and 80 ml 1 x TAE buffer (diluted from a 10 x concentrated stock solution using double distilled water) containing ethidium bromide (1 µg/ml) was added. The conical flask was covered with a foil cap and heated with a Bunsen burner to boiling point to dissolve the agarose completely. The dissolved agarose was allowed to cool to approximately 65 ^{O}C. The ends of the gel tray were sealed with autoclave tape. The cooled agarose was poured into the gel tray and tiny air bubbles removed using a sterile pipette tip. A comb was positioned in the agarose, which was then allowed to solidify.

Electrophoresis

An electrophoresis tank was filled with 1 x TAE buffer. The comb was carefully removed from the set agarose gel and the autoclave tape from the ends of the tray. The gel was then placed in the tank with care taken to ensure that the level of buffer in the tank was sufficient to cover the gel completely.

In a clean 0.5 ml microcentrifuge tube, a 5 µl aliquot of genomic DNA was added to 5 µl of PCR-grade water, with 2 µl of gel loading solution. Each preparation was loaded into the wells of the agarose gel. A 10 µl volume of *Hind*III DNA size standard marker was also loaded into one well with 2 µl of gel loading solution (to give final DNA conc.

0.02 µg/ml). The lid was placed on the tank, the power supply was connected, and electrophoresis was carried out at approximately 200 V and 100 mA until the dye front reached the end of the gel (approximately 1 hr).

Visualisation of DNA

The gel was transferred carefully onto the GelDoc System where the gel was illuminated with UV light and the resulting image printed onto thermal paper.

Determination of DNA yield and purity

DNA yield was determined by measuring absorbance readings of the extraction eluates at 260 nm. The spectrophotometer was set to measure absorbance at wavelengths 200-300 nm. Water (500 µl) was loaded into a quartz cuvette and used as a blank. The DNA preparation was then diluted 1 in 50 (10 µl in 490 µl double distilled water) and this 500 µl aliquot loaded into a quartz cuvette. Absorbance was recorded at 260 nm (DNA) and 280 nm (protein and other contaminants).

A concentration of 50 µg/ml DNA has an absorbance at 260 nm of 1. The DNA concentration in the eluate could therefore be calculated by multiplying the absorbance reading taken at 260 nm by 50 and then correcting for the dilution factor. The amount of the eluted DNA solution necessary to give the correction concentration of DNA required in the PCR reactions (1 µg of DNA) could then be calculated. The DNA extraction preparations were stored at -20 ^{O}C until ready for molecular analyses.

Preparation of primers for PCR

The primers required for *recA* amplification were the previously described BCR1 and BCR2 (Mahenthiralingam *et al.*, 2000a). An aliquot of 1 ml PCR grade water was added to each primer. It was known that 33 μg/ml of the primers had an absorbance at 260 nm of 1, the lengths of the primers BCR1 and BCR2 were 19 and 21 base pairs respectively, and the A_{260} values were 15.43 and 24.48 respectively. The concentration of the primers could therefore be calculated using the following equation:

$$\text{concentration of primers } (\mu M) = \frac{A_{260} \times 33 \times 10^3}{300 \times L \times 10^6} \times 10^6$$

(average nucleotide weight) (base pair length of primer)

The concentration of 1 ml of BCR1 was 89.3 μM, and 1 ml of BCR2, 128.2 μM. The desired concentration of primers for the *recA* PCR analysis was 25 μM, therefore a 1/3.57 dilution of BCR1 was prepared and a 1/5.13 dilution of BCR2.

PCR-based analysis

The following cycle was used to amplify the *recA* gene of Bcc strains:

window 1 – 95 ^{O}C (3 mins) 1 cycle

window 2 – 95 ^{O}C (2 mins)
58 ^{O}C (1 min) 35 cycles
72 ^{O}C (4 mins)

window 3 – 72 ^{O}C (20 mins) 1 cycle

A mastermix was prepared, the volume of which depending upon the number of samples run at any one time. This mastermix was prepared in water so that the final reaction conditions in each tube were as follows (44 µl):

0.5 µM of each primer

1 µl of 1 x PCR buffer

4 mM of $MgCl_2$

0.2 mM of each dNTP

2.5 U of Taq polymerase

1 µg template DNA

Preparation of a 1 % agarose gel was performed as described for DNA extraction.

Restriction enzyme digestion

The amplification product of each sample, the *recA* gene, was digested separately with two restriction enzymes, *Hae*III and *Mnl*I. It was found through trying various volumes of the amplification product in the digestion reactions (results not shown) that the best digestion results for visualization, were achieved when 8.4 µl amplification products (containing approximately 25 µg DNA) was incorporated into a 15 µl total volume reaction.

The following volumes were added to each reaction tube (1.5 ml microcentrifuge tube) for R.E digestion:

8.4 µl PCR product (approx. 25 µg DNA)

1.2 µl BSA (1mg/ml)

1.2 µl 10x buffer (*Hae*III or *Mnl*1 buffer)

1.2 µl *Hae*III (12 Units) or *Mnl*I (6 Units)

The tubes were incubated at 37 ^{O}C for 2 hrs.

Visualisation of DNA

A 13 cm by 15 cm gel consisting of 80 ml of 2.5 % (w/v) agarose was required. This was prepared as described for DNA extraction except 2 g agarose was placed in a clean 250 ml conical flask with 80 ml TAE buffer containing the ethidium bromide (1 µg/ml).

A 100 bp DNA size standard marker (5 µl) was loaded with 5 µl water and 2 µl gel loading buffer (to give final DNA conc. 0.02 µg/ml). A current of 100 mA (as used for separation of the extracted genomic DNA) was passed through the gel for 1.5 hrs, and the resulting gels photographed using the GelDoc system.

Interpretation of results

The BCCM control organisms were used to validate the reaction parameters, to allow accurate and reliable genomovar identification of the Bcc clinical isolates. This was achieved by comparison of *Hae*III and *Mnl*1 enzyme digestion patterns of the BCCM control strains with those of a panel of strains published by McDowell *et al.* (2001). Following this, the 112 Bcc clinical isolates were analysed in an attempt to determine their genomovar status.

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed by the Dept. of Medical Microbiology, Teviot Place, University of Edinburgh, UK. One Bcc strain isolated from each patient was analysed, unless both pre- and post-transplant strains were available. The following protocol was adopted:

All Bcc strains were blinded and genotyped by macrorestriction of whole genomic DNA followed by PFGE. Organisms were grown overnight at 37 ^{O}C on nutrient agar. The organisms were then harvested, washed in 1 ml of 75 mM NaCl solution containing 25 mM EDTA, and centrifuged. The pellet was then re-suspended in 500 µl of the same buffer and mixed with 500 µl of 1.0 % (w/v) molten low-melt agarose before addition to

an insert mold. Bacteria were lysed by incubating the agarose plugs with a 1 % (w/v) solution of *N*-laurylsarcosine, containing 0.5 M EDTA and 0.5 % (vol/vol) Triton X-100. An additional proteinase K digestion step (0.05 mg/ml) was required for suspected `epidemic` ET-12 strains. Genomic DNA was digested with the restriction enzymes *XbaI* and/or *Spe*I and fragments separated by PFGE (CHEF DRII system; Bio-Rad) with pulse times of 2.9 to 35.4 sec at 6 V/cm for 20 hrs at 14 ^{O}C. Gels were stained with ethidium bromide and viewed under UV light. Genotype patterns for *B. cenocepacia* isolates were compared with the ET-12 index strain J2315 using published guidelines that take account of minor band differences that occur between clonally-related isolates (Tenover *et al.*, 1995).

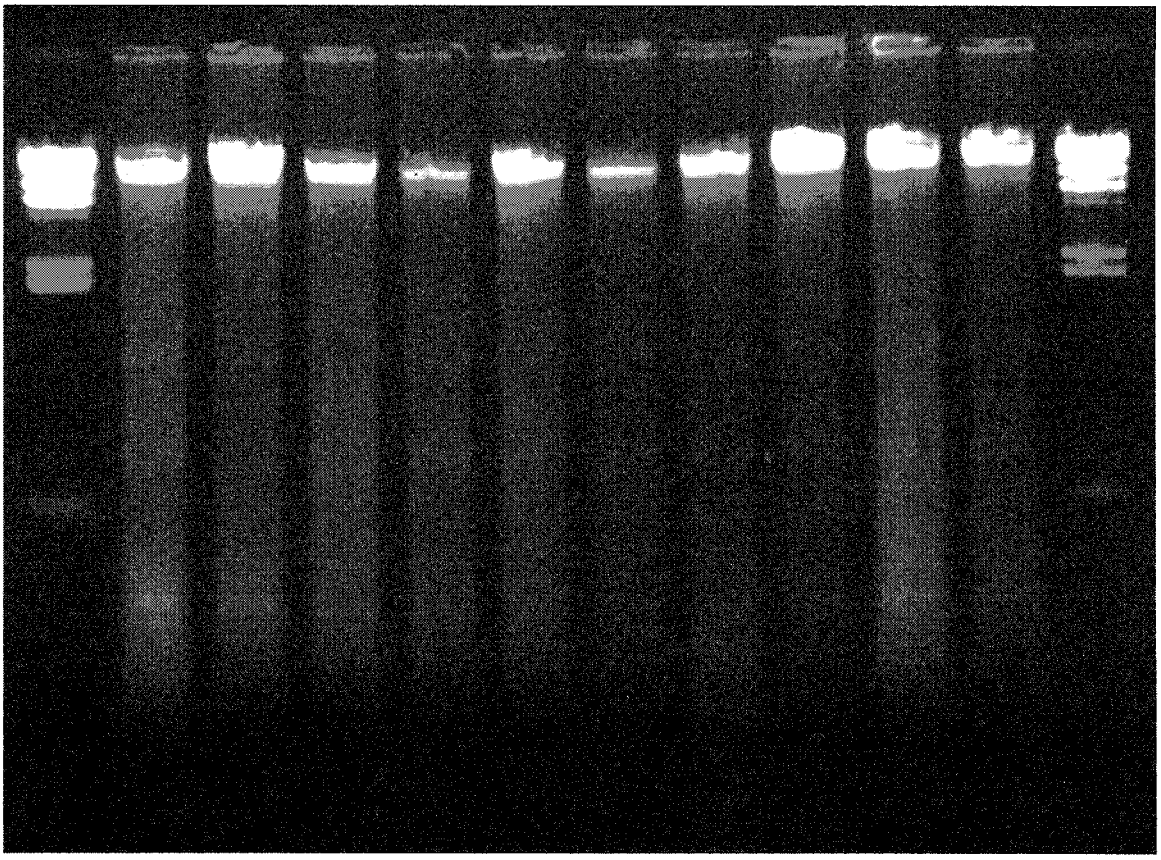
Results

RecA gene analysis of the Bcc control strains and clinical isolates

The genomes were successfully extracted from all 30 BCCM control and 112 Bcc clinical isolates. Visualization of the DNA in agarose gel alongside a Lambda *Hind*III ladder demonstrated the size of the genomes to be around 8 Mb (genome size of *B. cepacia* type strain ATCC 25416). Figure 2.3 shows a representative sample of extracted genomes from Bcc control strains and clinical isolates. Table 2.1 shows the absorbance values, and subsequently the DNA concentrations, of these extractions.

The absorbance readings at 260 nm were all between 0.1 and 1.0, the absorbance required to allow for accurate readings. The yield of DNA was approximately 340 µg per 2 ml overnight bacterial growth for all extractions. Therefore 6 µl of each extraction was required to give approximately 1 µg of DNA (the amount required for the 50 µl PCR reactions). This amount of template DNA was incorporated into all PCR reactions performed.

Figure 2.3: Representative sample of extracted genomes of 2 control and 8 wild Bcc strains to include genomovars I-V



L	C1	W13	W28	C23	W2	W19	W32	W36	W55	W100	L
Genomovar:	*I*	*III-A*	*II*	*IV*	*V*	*II*	*II*	*III-A*	*III-A*	*III-B*	

Key to strains:

L, Lambda *Hind*III ladder; C1, LMG1222; C23, LMG 14086; for wild strains (W#) see Appendix 2.1.

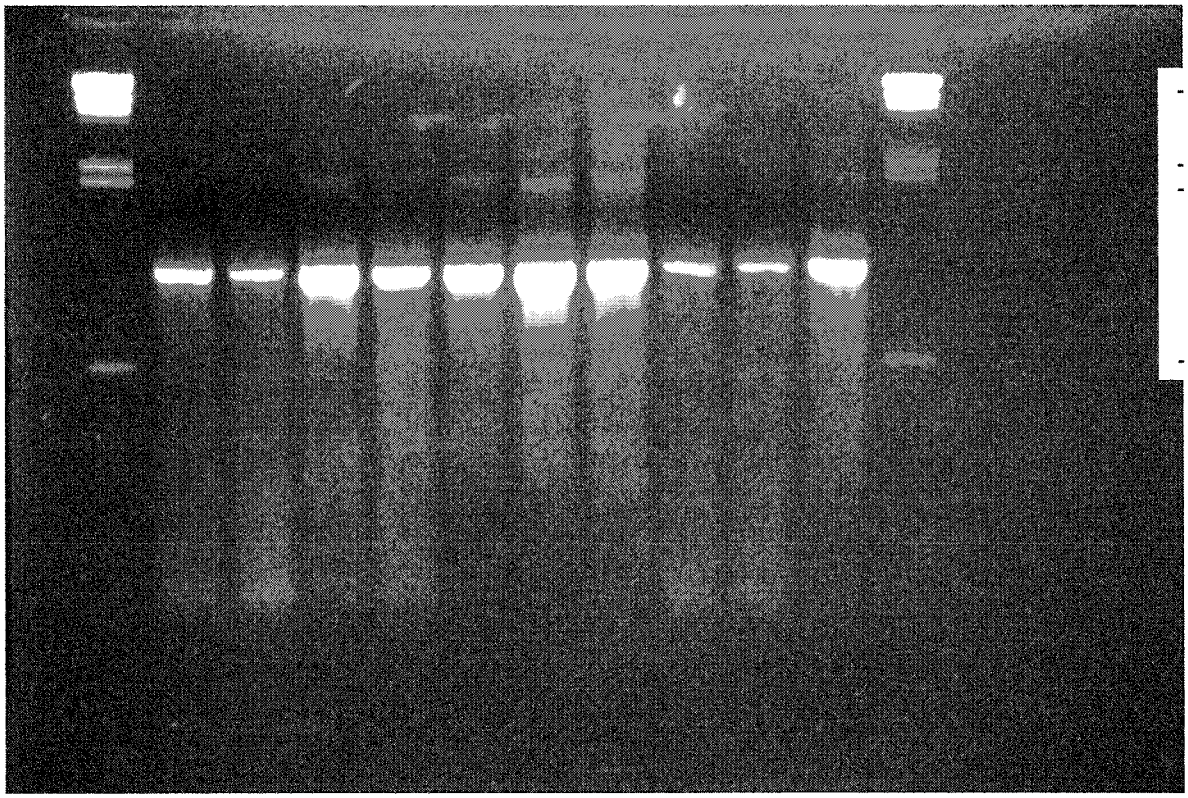
Table 2.1: A_{260} readings, DNA concentration, and DNA/protein ratio of Belgian Co-ordinated Collections of Micro-organisms (BCCM) Bcc strains

Strain no.	Reference	Genomovar	Wavelength	A_{260}	DNA concentration (µg/µl)	Ratio (260/280)
C1	LMG 1222	I	260	0.067	0.1675	1.20
			280	0.056		
W13	Mb 864	III-A	260	0.077	0.1925	1.28
			280	0.06		
W28	Mb 2384	II	260	0.061	0.1525	1.17
			280	0.052		
C23	LMG 14086	IV	260	0.058	0.145	1.18
			280	0.049		
W2	Mb 917	V	260	0.064	0.160	1.21
			280	0.053		
W19	Mb 1987	II	260	0.059	0.148	1.26
			280	0.047		
W32	Mb 1488	II	260	0.063	0.158	1.21
			280	0.052		
W36	Mb 1148	III-A	260	0.075	0.188	1.29
			280	0.058		
W55	Mb 2295	III-A	260	0.075	0.1875	1.39
			280	0.054		
W100	2952795	III-B	260	0.074	0.185	1.10
			280	0.067		

The Bcc *recA* gene is just over 1 Kb in size (Mahenthiralingam *et al.*, 2000a). Figure 2.4 shows the resulting amplicon following PCR of the *recA* gene from the same representative sample of Bcc strains, the genomic DNA of which were visualized in

Figure 2.3. Only one band was present per strain indicating that the *recA* gene alone was amplified.

Figure 2.4: Representative sample of amplified 1kb *recA* genes of 2 control and 8 wild Bcc strains to include genomovars I-V



	L	C1	W13	W28	C23	W2	W19	W32	W36	W55	W100	L
Genomovar:		*I*	*III-A*	*II*	*IV*	*V*	*II*	*II*	*III-A*	*III-A*	*III-B*	

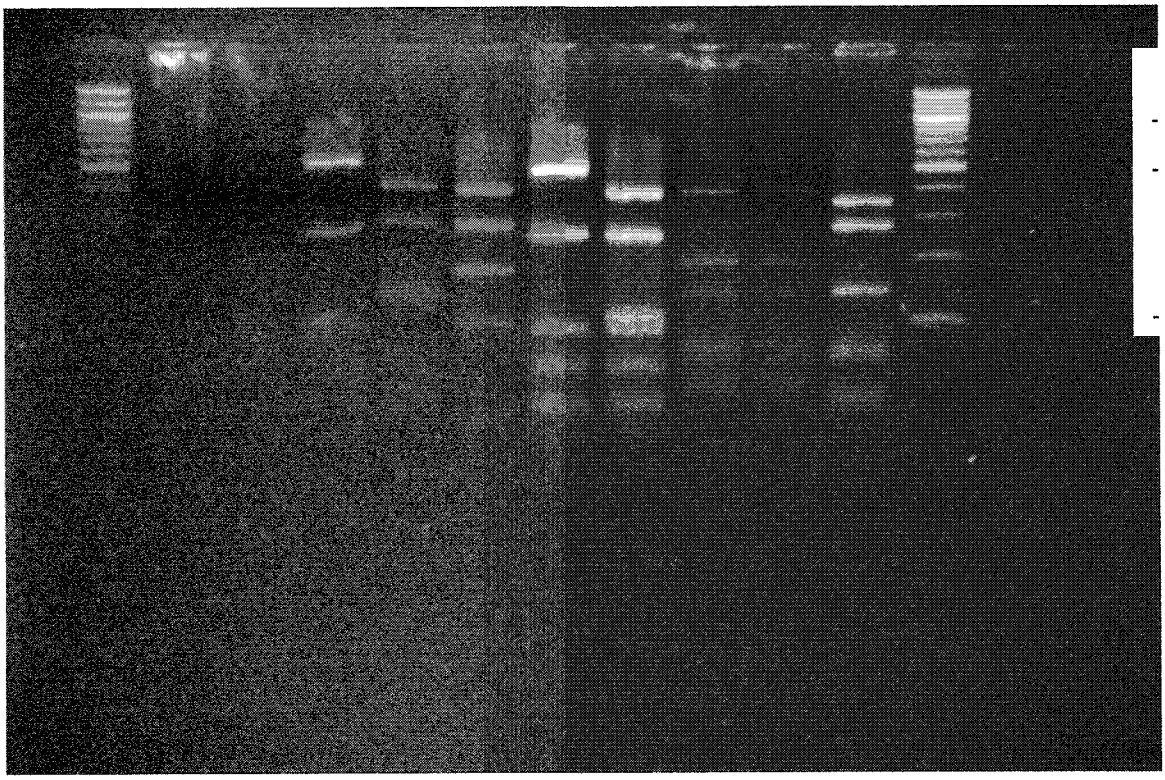
Key to strains:

L, Lambda HindIII ladder; C1, LMG1222; C23, LMG 14086; for wild strains (W#) see Appendix 2.1.

By comparing the *Hae*III digestion patterns of the control strains to the base pair ladders, and the patterns published by McDowell *et al.* (2001) it could be confirmed that the technique had worked successfully and the genomovars were correctly identified.

Using the second enzyme, *Mnl*I concomitantly with *Hae*III ensured that the genomovar allocation was correct. The genomovars of the clinical isolates were then determined using the same comparisons. Figures 2.5 and 2.6 show the *Hae*III and *Mnl*1 digestion products of the *recA* gene from the same representative sample of Bcc control and wild strains. A total of 109 of the 112 wild strains from the different patients were successfully typed using *recA* RFLP analysis (see Appendix 2.2). There were no amplification products from wild strains W30, W31 and W81. It was therefore concluded that these strains had been misidentified by phenotypic methods and were in fact not members of the Bcc.

Figure 2.5: Representative sample of *Hae*III digestion of 2 control and 8 wild Bcc strains to include genomovars I-V

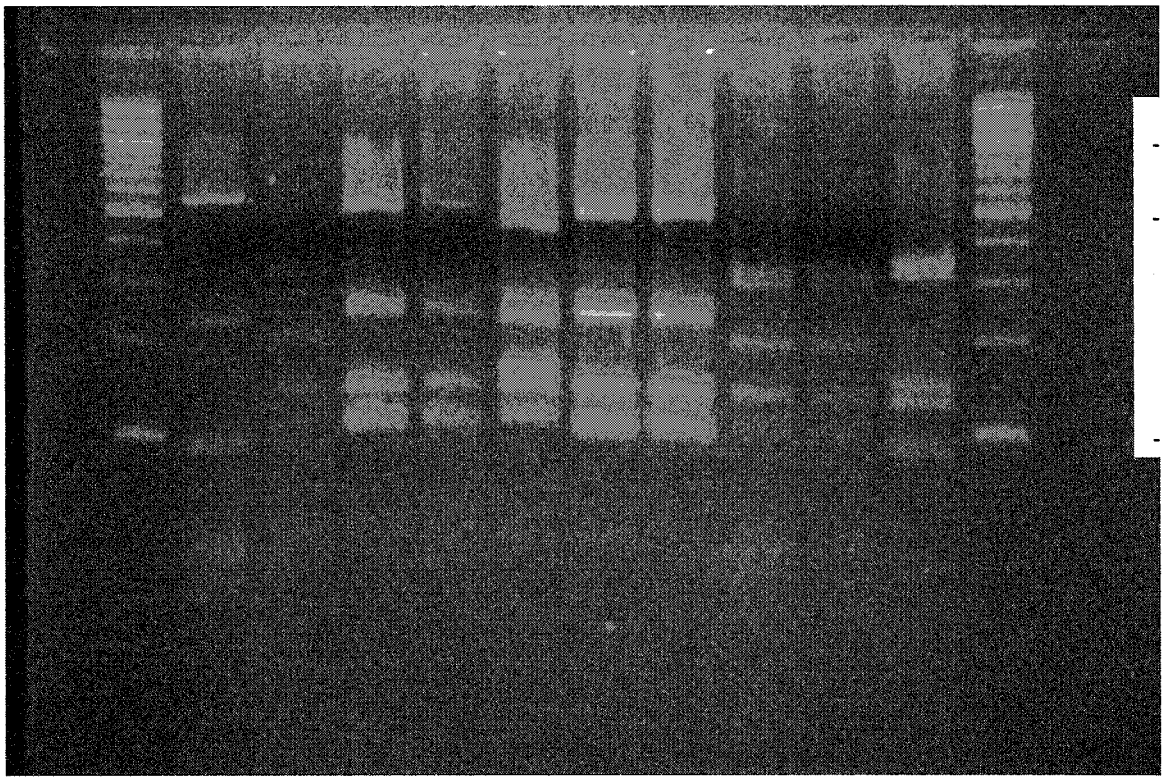


	L	C1	W13	W28	C23	W2	W19	W32	W36	W55	W100	L
Genomovar:		*I*	*III-A*	*II*	*IV*	*V*	*II*	*II*	*III-A*	*III-A*	*III-B*	
RE pattern:		*E*	*G*	*F*	*J*	*A*	*F*	*F*	*G*	*G*	*H*	

Key to strains:

L, Lambda *Hind*III ladder; C1, LMG1222; C23, LMG 14086; for wild strains (W#) see Appendix 2.1

Figure 2.6: Representative sample of *MnlI* digestion of 2 control and 8 wild Bcc strains to include genomovars I-V



	L	C1	W13	W28	C23	W2	W19	W32	W36	W55	W100	L
Genomovar:		*I*	*III-A*	*II*	*IV*	*V*	*II*	*II*	*III-A*	*III-A*	*III-B*	
RE pattern:		*e*	*f*	*a*	*b*	*c*	*a*	*a*	*f*	*f*	*h*	

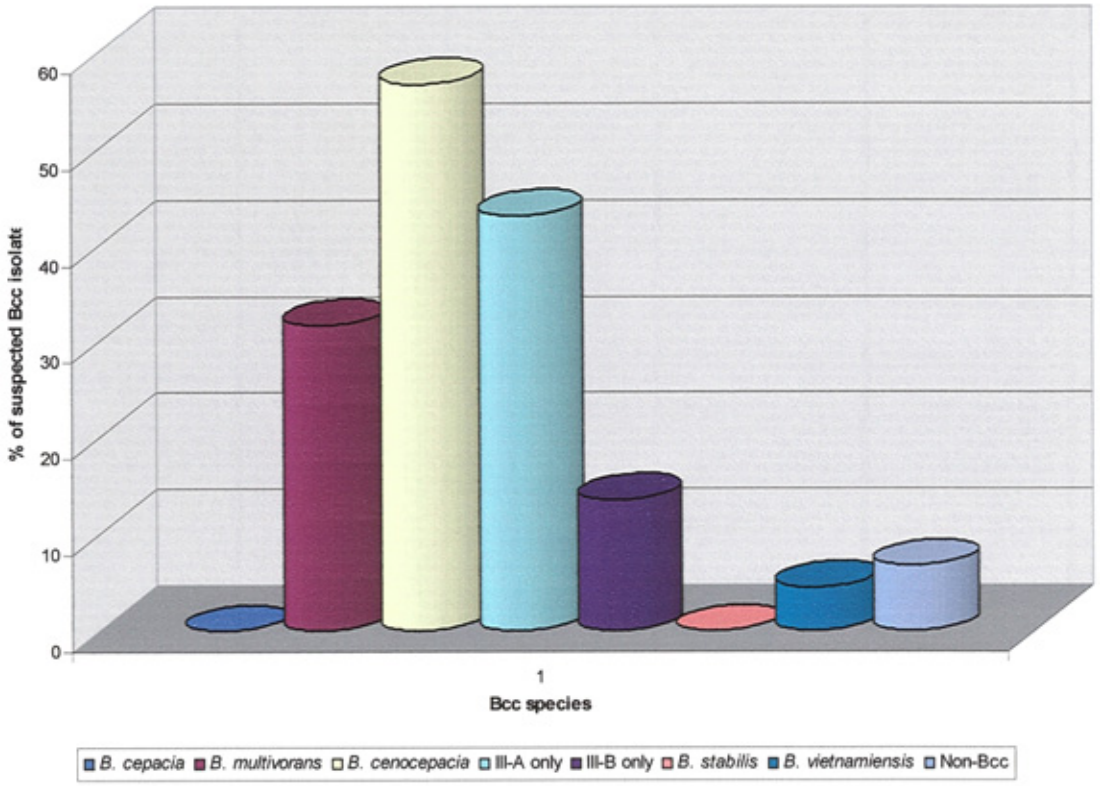
Key to strains:

L, Lambda *Hind*III ladder; C1, LMG1222; C23, LMG 14086; for wild strains (W#) see Appendix 2.1

Prevalence of Bcc genomovars in CF patients referred to the Freeman Hospital Transplant unit

A total of 44 patients were initially found to have presumptive Bcc infection as assessed by growth on selective agar and phenotypic analysis of the resulting isolates. Of the three isolates for which PCR-based molecular analysis revealed were not organisms of the Bcc, one isolate was identified as *Brevundimonas vesicularis,* while isolates from two patients were identified as *Alcaligenes xylosoxidans*. Twenty five of the remaining 41 patients were found to be colonised with *B. cenocepacia* (57 %). Nineteen patients (43 %) were colonised with the *recA* lineage III-A, while 6 patients (14 %) were colonised with the III-B lineage. A total of 14 patients (32 %) were infected with *B. multivorans*, while the remaining 2 patients (5 %) had isolates of *B. vietnamiensis*. The prevalence of genomovars is illustrated in Figure 2.7.

Figure 2.7: Frequency of genomovar status in Bcc wild strain collection (total 44)



Molecular Epidemiology of *B. cenocepacia* infection

Strains from 32 patients were analysed by PFGE (see Appendix 2.3). Sixteen of these patients were infected with *B. cenocepacia*. Four strain types were found among the 16 patients tested who were infected with *B. cenocepacia* (see Appendix 2.3). A total of 13 patients were infected with the highly transmissible ET-12 epidemic strain of *B. cenocepacia* III-A (i.e. 81 % *B. cenocepacia* III-A isolates) All ET-12 isolates contained both the *cblA* gene and BCESM. One patient was infected with a unique *B. cenocepacia*

III-A strain that lacked the c*blA* gene but possessed the BCESM (work performed by the Biomolecular Sciences Group, Queen's University, Belfast). Both *B. cenocepacia* III-B-infected patients harbored genetically unique strains that were BCESM and c*blA* negative. Figure 2.8 shows the macrorestriction (*Spe*I) fingerprinting of the Bcc genomovar strains. Lane 4 contains the unique *B. cenocepacia* III-A strain, lanes 5-6, the unique *B. cenocepacia* III-B strains, lanes 9-12, a representative sample of *B. cenocepacia* III-A ET-12 isolates and lane 13, J2315 Edinburgh index strain for ET-12 lineage.

Figure 2.9 demonstrates the persistence of pre-transplant Bcc strains in post-transplant cultures by macrorestriction (*Spe*I) fingerprinting. PFGE analysis of pre and post-transplant ET-12 isolates from 3 representative patients revealed the persistence of the epidemic strain after surgery. Lanes 3-4 contain pre- and post-transplant ET-12 strains from patient C, lanes 5-6, from patient U and lanes 7-8 from patient M.

Molecular epidemiology of *B. multivorans* and *B. vietnamiensis* infections

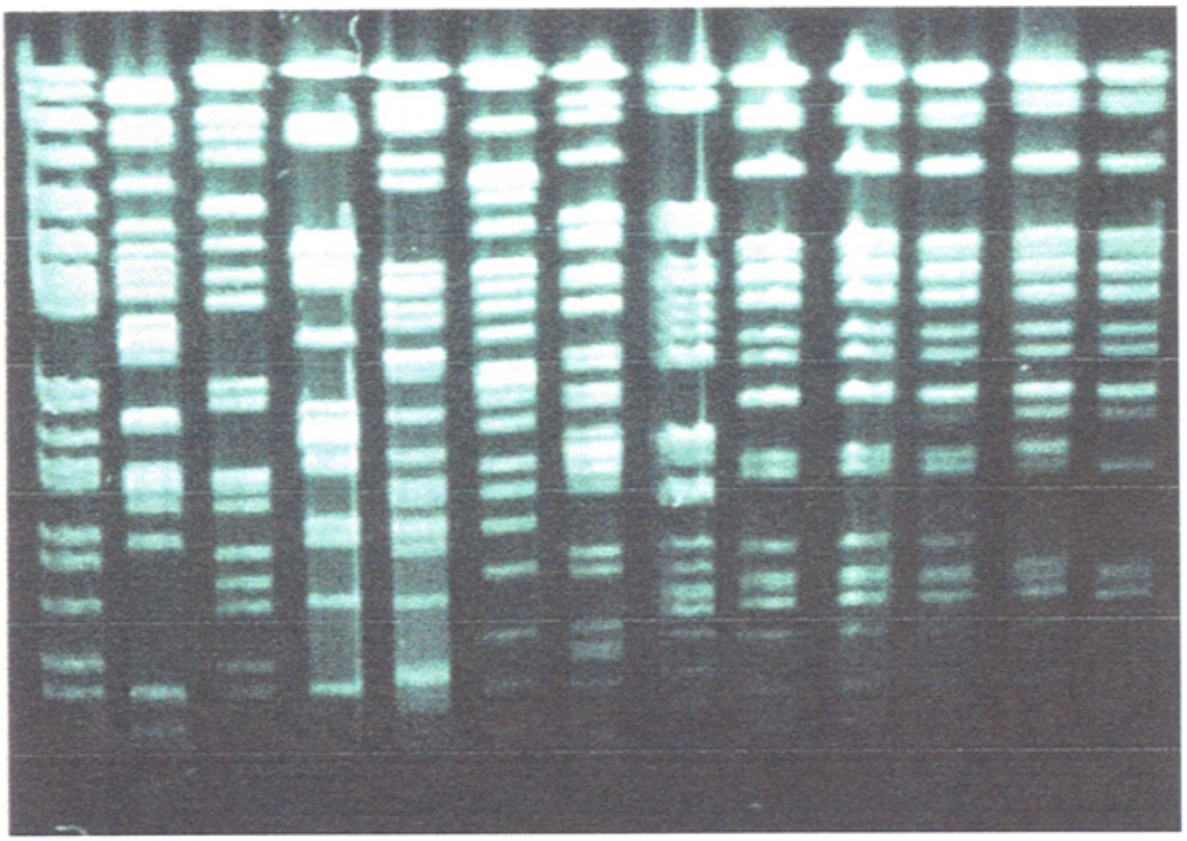
All patients infected with *B. multivorans* and *B. vietnamiensis* were infected with unique strains. Samples of the genetic fingerprints obtained by PFGE for these strains are shown in Figure 2.8. Lanes 1 to 3 contain a representative sample of unique *B. multivorans* strains, lanes 7-8, the unique *B. vietnamiensis* strains. All these strains were negative for the C*blA* gene and BCESM. PFGE analysis of pre and post-transplant isolates from one representative patient with *B. multivorans*, and both patients with *B. vietnamiensis* again revealed the persistence of the infecting strain after surgery (see

Figure 2.9). Lanes 1-2 contain pre- and post-transplant isolates from patient K (*B. multivorans*), lanes 9-10, from patient B (*B. vietnamiensis*) and lanes 11-12, from patient E (*B. vietnamiensis*).

Strain types and post-transplant outcomes

This study has demonstrated that the previously described post-transplant deaths from the Freeman Hospital Transplant Unit (De Soyza *et al.*, 2001) were associated with the ET-12 epidemic strain of *B. cenocepacia*. One further patient infected with the ET-12 strain for at least 3 years died of "cepacia syndrome" while on the active transplant waiting list.

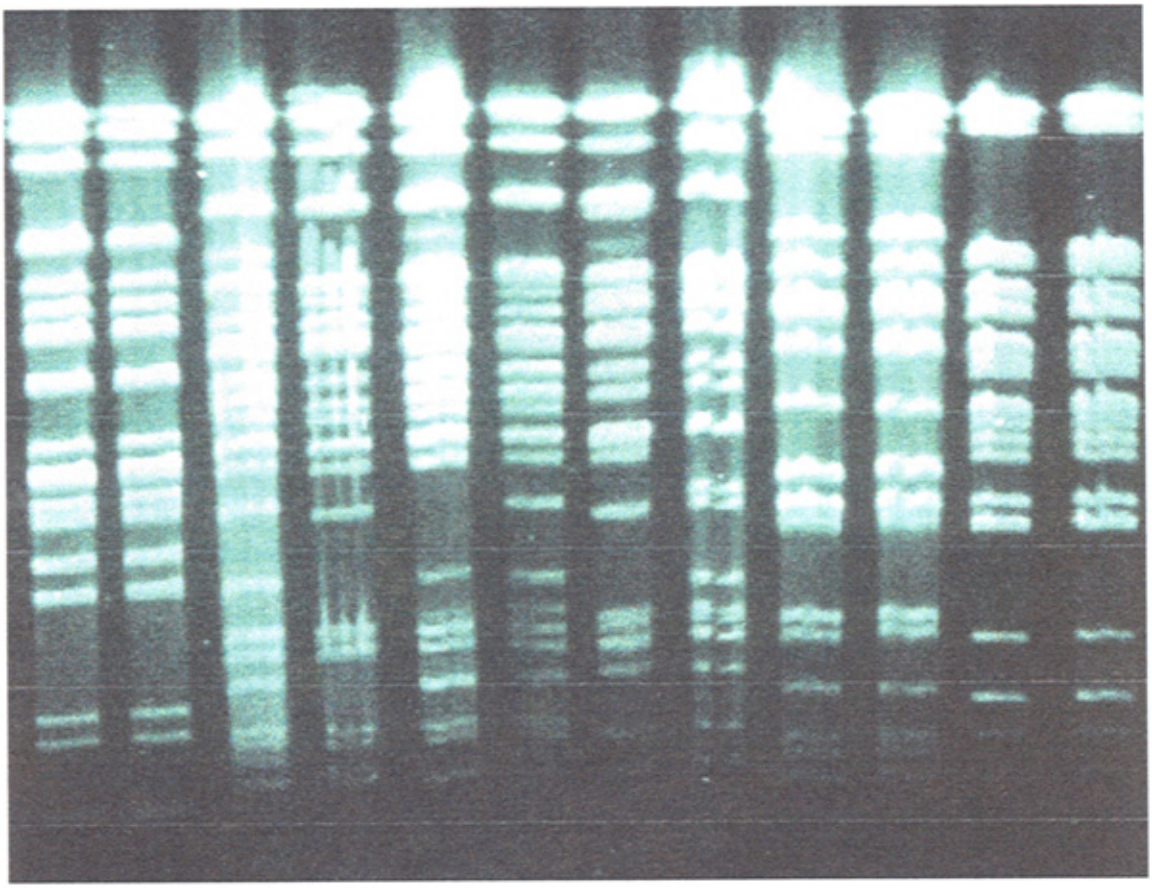
Figure 2.8: Macrorestriction (*Spe*I) fingerprinting of Bcc strains recovered from CF patients attending the Freeman Hospital Transplant Unit



II II II III-A III-B III-B V V III-A III-A III-A III-A J2315

ET-12

Figure 2.9: Demonstration of the persistence of pre-transplant Bcc strains in post-transplant cultures by macrorestriction (*Spe*I) fingerprinting.



Discussion

This study describes an extensive epidemiological investigation to determine the prevalence and clonality of Bcc isolates recovered from CF patients (1989 to present) referred for pre-transplant assessment. This allowed us to understand the nature and emerging pattern of Bcc infections amongst these patients, and also provided valuable information for downstream post-transplant management of individual patients based on their Bcc genomovar and strain types.

Genomovar distribution of Bcc isolates

Selective culture and phenotypic analysis identified 44 patients with Bcc infections. However, PCR-based molecular analyses revealed only 41 patients were in fact colonised with Bcc pathogens. Problems associated with the use of phenotypic tests as the sole means of identifying Bcc bacteria has been described previously (Kiska *et al.*, 1996; Coenye *et al.*, 2000; McMenamin *et al.*, 2000; Henry *et al.*, 2001; McDowell *et al.*, 2001; Moore *et al.*, 2002). False positive rates of identification highlight the importance of molecular analyses for accurate identification of Bcc organisms, particularly in the context of pre-transplant assessment.

Our present study found that the majority of Bcc-infected patients referred to the Freeman Hospital Transplant Unit were infected with *B. cenocepacia* (57 %), followed by *B. multivorans* (32 %), then *B. vietnamiensis* (5 %). There were no strains of *B.*

cepacia or *B. stabilis* isolated (see Figure 2.7). In agreement with our findings, other studies have reported similar genomovar distributions within the CF population. In particular that although all Bcc species have been recovered from CF sputum culture, *B. multivorans* and *B. cenocepacia* (in particular *recA* lineage III-A) account for the majority of CF isolates, and that most epidemic Bcc isolates are *B. cenocepacia* III-A and that this species is associated with greater morbidity and mortality (Gillis *et al.*, 1995; Vandamme *et al.*, 1997; LiPuma *et al.*, 1999; Mahenthiralingam *et al.*, 2000a).

There were no RFLP patterns found in this study upon restriction of the amplified *recA* gene with *Hae*III and *Mnl*1, which did not match the existing patterns documented by McDowell *et al.* (2001) for genomovars I-V. As genomovars VI-IX have been reported to give *Hae*III and *Mnl*1 digestion bands which differ to these previously established patterns, it can be concluded that non of the CF patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit were colonized with any of these more recently described genomovars (Coenye *et al.*, 2001a, 2001b; Mahenthiralingam *et al.*, 2002; Vandamme *et al*, 2002).

Molecular epidemiology of Bcc strains

Pulmonary transplantation has emerged as a highly successful treatment for end stage CF-associated lung disease. However, there has been much debate regarding the role of transplantation in those infected with Bcc reflecting the variable outcomes seen in these patients (reviewed in Husain and Singh, 2002). Many transplant units consider pre-transplant infection with Bcc an absolute contraindication for surgery. Currently,

prevalence rates of Bcc infection amongst CF patients transplanted at the Freeman Hospital Transplant Unit is 15 % (De Soyza *et al.*, 2001). This incidence of pre-transplant infection is comparable to the reported levels of 22 % observed in five other UK transplant centres (Egan *et al.*, 1994). De Soyza *et al.* (2001) previously found that poor-transplant outcomes were associated with pre-transplant *B. cenocepacia* infections, while infections with other genomovars were associated with excellent post-transplant prognoses, thus providing insights into the variable outcomes previously observed in transplant patients in North Carolina, United States with "*B. cepacia*" infections (Aris *et al.*, 2001). Data from both these studies provided evidence that the moratorium placed on all Bcc-infected CF patients by many transplant units was not justified, particularly with the availability of recently described molecular-based diagnostic tests that could discriminate *B. cenocepacia* from other *B. cepacia* complex genomovars associated with better outcomes (Mahenthiralingam *et al.*, 2000a, 2002).

Analysis of these 29 isolates by PFGE revealed that 13 patients, referred from geographically diverse CF centres, were infected with the ET-12 epidemic clone of *B. cenocepacia* III-A, while 1 patient harbored a unique *B. cenocepacia* III-A strain. The origins of the ET-12 lineage are unclear. It may have first infected patients in Toronto before spreading across Canada, and subsequently there may have been transatlantic introduction into the UK CF population as a consequence of social contact at CF summer camps (Govan *et al.*, 1996). In contrast to the UK, the ET-12 strain is found infrequently amongst CF patients in the United States, although the spread of other *B. cenocepacia* strains, such as PHDC and the "Midwest" clone have been described (Chen *et al.*, 2001, Kumar *et al.*, 1997).

Our genotyping studies have also revealed that all our previously described Bcc-related post-transplant deaths were associated with the ET-12 strain of *B. cenocepacia* III-A. This contrasts with the recent North Carolina transplant study, which found that five post-transplant deaths were associated with unique *B. cenocepacia* III-A genotypes, all lacking the *cblA* gene (Aris *et al.*, 2001). Small clusters of cross-infection involving two genotypically distinct *CblA*-negative *B. cenocepacia* strains were also observed, further revealing the heterogeneous nature of *B. cenocepacia* infections amongst this group of patients, and the absence of the ET-12 epidemic strain compared to our own patient cohort. A separate study from the same centre found that amongst 56 Bcc-infected patients assessed for transplantation, the majority harbored strains with unique genotypes, providing further evidence for the heterogeneous nature of Bcc infections amongst CF patients referred to this transplant unit (Heath *et al.*, 2002). Only three patients were found to be infected with *CblA*+ strains. The North Carolina transplant study observed that all *B. multivorans*-colonised patients (n=7) harbored unique strains revealing sporadic infection (Aris *et al.*, 2001). We similarly found all our *B. multivorans* strains, as well as our *B. vietnamiensis* strains and both *B. cenocepacia* III-B strains, to be genotypically distinct.

During this study, genotyping analysis also revealed that the pre-transplant strain remains responsible for post-transplant infections, confirming the previous study of Steinbach *et al.* (1994). This persistent infection may reflect, in part, the great difficulty in clearing the fused pleural spaces often seen intra-operatively. An alternative explanation for the persistence of the pre-transplant strain may be on-going para-nasal infection leading to infection of the graft.

In conclusion, the development of these novel molecular tools has provided the scientific community with quick, easy, and scientifically sound ways of identifying individual strains belonging to this taxonomically complex group of organisms. The *recA* PCR-RFLP analysis is a reliable molecular technique for Bcc genotyping, in particular genomovars I-V. Applying this technique to 112 Bcc strains from a total of 44 CF patients referred to the Freeman Hospital Transplant Unit demonstrated *B. cenocepacia* III-A and *B. multivorans* to be the predominant colonizing Bcc genomovars in these patients. Despite a geographically wide referral base from many different CF centres, infection with the ET-12 epidemic clone was the most prevalent strain amongst *B. cenocepacia*-colonised patients referred to the Freeman Hospital Transplant Unit for pre-transplant assessment. Although the incidence of Bcc infection has declined as a consequence of strict segregation policies in CF centres in North America and the UK, studies such as ours confirm that transmission can still occur between Bcc infected and non-infected patients who congregate socially (Muhdi *et al.*, 1996). In addition, environmental sources may also contribute to new acquisitions of Bcc infections (Balandreau *et al.*, 2001; LiPuma *et al.*, 2002). Therefore, there is still likely to be a significant number of patients infected with organisms of the Bcc who will require transplantation in the future.

CHAPTER 3

Evaluation of enzyme substrates for differentiation of the Bcc from closely related organisms and discrimination between the individual species of the Bcc

Introduction

As previously discussed, it is possible to determine the genomovar status of *B. cepacia* complex (Bcc) strains by using various molecular techniques. These include DNA-DNA hybridization (Vandamme *et al.*, 1996, 1997) restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA PCR product, RFLP analysis of the *recA* gene, and genomovar-specific *recA* PCR, (LiPuma *et al.*, 1999; Mahenthiralingam *et al.*, 2000a; Henry *et al.*, 2001). The development of these novel molecular tools has provided the scientific community with reliable and scientifically sound ways of identifying individual strains belonging to this taxonomically complex group of organisms. However, these methods are laborious and technically demanding, and moreover, PCR primers are not available for all the genomovars, nor indeed, for all organisms closely related to members of the Bcc, and therefore not suitable for routine diagnostic microbiology laboratories. Reliable, user friendly and cost effective phenotypic tests would be a preferable alternative to such tests. Although extensive phenotypic tests such as whole cell protein profile analysis, can be applied to assist in genomovar identification, due to their high degree of phenotypic similarity, simple biochemical studies alone cannot yet reliably classify the nine species collectively referred to as the Bcc, nor distinguish them from closely related organisms such as *Burkholderia gladioli, Pandoraea* sp. and *Ralstonia pickettii* (Henry *et al.*, 2001). *Pandoraea*, for example, is a recently described genus for which there are many problems associated with laboratory identification (Coenye *et al.*, 2000; Moore *et al.*, 2002b). Due to the marked differences in apparent pathogenicity and prevalence among the genomovars, a simple phenotypic scheme for classification is needed.

The use of enzyme substrates to detect enzymes within bacterial species is a possible alternative to these extensive phenotypic tests. Such enzyme substrates are commonly applied to various bacterial species for identification purposes with great success (Berdal and Olsvick, 1983; Kämpfer *et al.*, 1991; Manafi *et al.*, 1991; Wauters *et al.*, 1995; Manafi, 1996). Every bacterial species has a unique profile, a set of enzymes required for growth, nutrition and replication, and this differential distribution of enzymes among microbial species provides a convenient method for identification (Bascomb, 1987; Manafi *et al.*, 1991; Manafi, 1996; de Boer, 1998). Such enzymes may be conveniently assayed using a suitably labelled substrate and a sensitive detector of the cleaved label. The application of enzyme substrates as diagnostic tools dates back as far as 1907 when a report described the use of esculin (a natural enzyme substrate) to identify pathogenic bacteria within the Enterobacteriaceae (Ter Meulen; 1907, cited in Trepeta and Edberg, 1987). Esculin production has since been investigated in numerous other bacterial species, including Bcc strains (Wauters *et al.*, 1995, Henry *et al.*, 2001).

A number of studies have been performed to elucidate enzyme profiles for a wide range of bacterial species, consequently these qualitative enzyme tests are useful for differentiating between various bacteria (Westley *et al.*, 1967; Grange *et al.*, 1979; Godsey *et al.*, 1981; Feng and Hartman, 1982; Berdal and Olsvick, 1983; Trepeta and Edberg, 1987; Kämpfer *et al.*, 1991; Manafi *et al.*, 1991; Dealer *et al.*, 1993; James, 1994; Bitton *et al.*, 1995). The majority of published work in this area has concerned itself with the differentiation of bacteria for diagnostic purposes (Mulczyk *et al.*, 1970; Gupta *et al.*, 1974; Giammanco *et al.*, 1982, Kämpfer, 1992). The relative rapidity and ease of applying such tests has led to their wide usage, particularly in kit form, for identification purposes such as the API 20NE (API-bioMérieux, La Balme les Grottes,

France), widely used today for the differentiation and identification of non-enteric Gram-negative rods. A limited number of studies has been performed to elucidate the enzyme profiles of Bcc strains and related organisms such as *Pandoraea* sp. and *R. pickettii,* in particular, whether the nine species comprising the Bcc can be differentiated using such profiles (Welch *et al.*, 1987; Kämpfer and Dott, 1988; Perry *et al.*, 1998; Henry *et al.*, 2001; Laffineur *et al.*, 2002;). The Crystal Enteric/Non-Fermenter ID kit is an identification system based on 30 biochemical tests, including enzyme detection, which has been used to obtain the enzyme profiles for a number of species, including "*B. cepacia*". The kit screens for a number of enzymes, including phosphatase, ß-glucosidase and ß-xylosidase (Holmes and Howard, 1994; Wauters *et al.*, 1995).

Many chromogenic and fluorogenic enzyme substrates have been developed, all of which follow the same basic principle of activity. Brightly coloured (chromogenic) or highly fluorescent (fluorogenic) core molecules are linked, typically via a hydroxyl residue, to a particular metabolic moiety e.g. glucose or phosphate, to form colourless or non-fluorescent enzyme substrates. Subsequent hydrolysis by the appropriate bacterial enzyme releases the core compound and restores the bright colour or the intense fluorescence.

Fluorogenic enzyme substrates

Dyer (1970) was the first to suggest the use of fluorogenic substrates for the detection of bacterial enzymes. The most commonly used fluorogenic substrates are based on the fluorescent core molecule coumarin (see Figure 3.1). These include 4-methylumbelliferone (MU; 7-hydroxy-4-methylcoumarin) or 7-amino-4-

methylcoumarin (7AMC), the structures of which are shown in Figure 3.2. These coumarinic compounds have, like other fluorophores, structural features which predispose towards fluorescence. These are planarity, molecular rigidity, electron delocalization via an efficient conjugated system and the presence of at least one electron-releasing group (James, 1994).

Figure 3.1: Structure of fluorescent core molecule coumarin

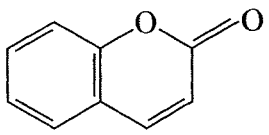
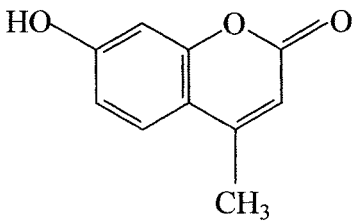
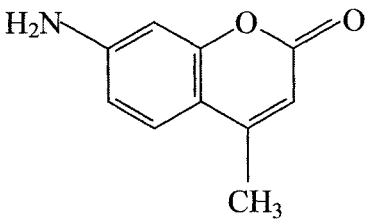


Figure 3.2: Structures of (i) 4-methylumbelliferone (7-hydroxy-4-methylcoumarin) and (ii) 7-amino-4-methylcoumarin



(i) 4-methylumbelliferone (MU)



(ii) 7-amino-4-methylcoumarin (7AMC)

When MU is conjugated to a metabolically active moiety (such as glucose), the substrate is non-fluorescent. On hydrolysis, the aglycone is cleaved from the glucose, freeing up

the electrons at the hydroxyl group and altering the molecule such that it yields a bright blue fluorescence under UV light, i.e. at an excitation wavelength of 365 nm and emission wavelength of 445 nm (Haughland, 1996). This blue fluorescence can be observed visually or detected fluorometrically when plates, tubes or wells are irradiated with long-wavelength UV light.

Figures 3.3 and 3.4 illustrate two such commonly used fluorogenic enzyme substrates, alanine linked to 7AMC (L-alanyl-AMC), and galactose linked to MU (MU-ß-galactoside) respectively, and the resulting fluorescent molecules after hydrolysis.

Figure 3.3: Hydrolysis of non-fluorescent L-alanyl-7-amido-4-methylcoumarin (L-alanyl- AMC) to fluorescent 7AMC

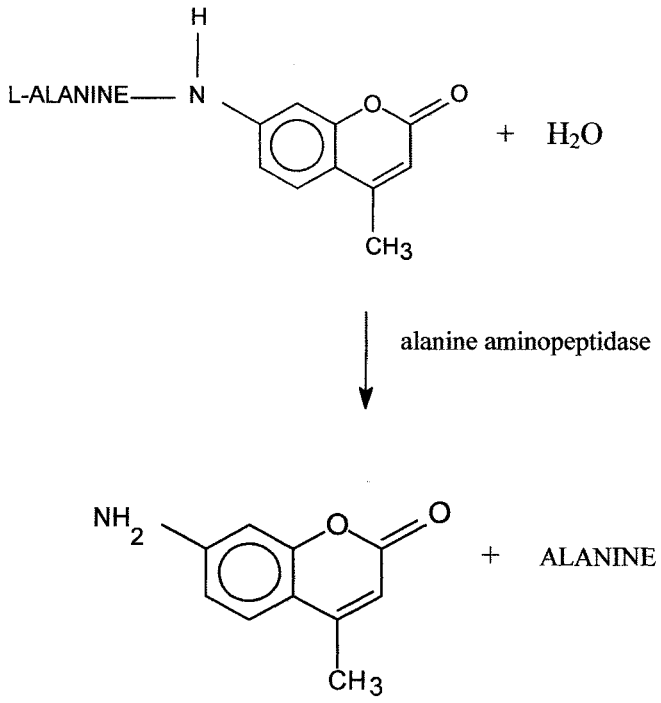
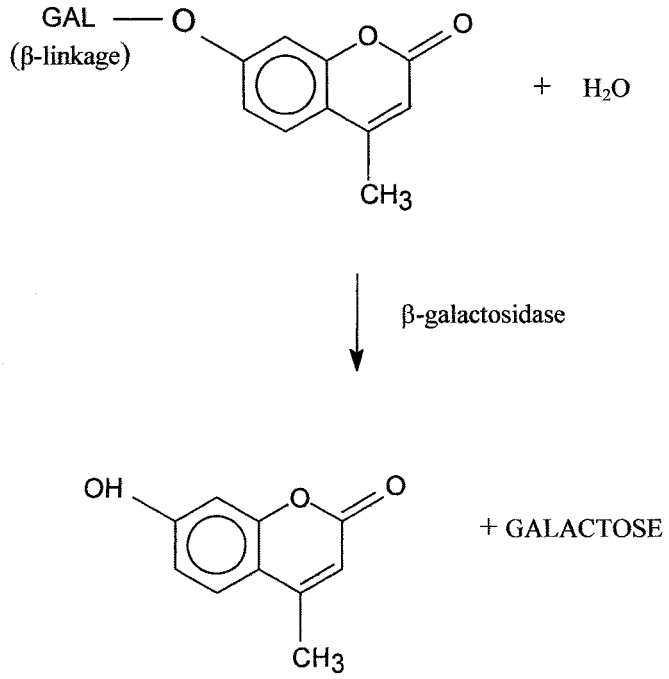


Figure 3.4: Hydrolysis of non-fluorescent 4-methylumbelliferyl-ß-D-galactoside (MU-ß-galactoside) to fluorescent MU



Fluorogenic synthetic enzyme substrates containing coumarin derivatives of MU or 7AMC have been used extensively for the detection of enzymes in diagnostic microbiology (Trepeta and Edberg, 1984; Manafi *et al.*, 1991; Dealer *et al.*, 1993; James, 1994; Bitton *et al.*, 1995). This popularity could be ascribed to availability of a wide range of substrates with different metabolic moieties, non-carcinogenicity, ease of hydrolysis, intense fluorescence generated on release of the fluorescent molecule, ease of visual detection of the products of enzyme activity with UV light sources, and availability of suitable fluorometers for measurement of fluorescence in both tube and multiwell panels (Shadix and Rice, 1991; Brenner *et al.*, 1993). An important feature of any coumarin-based substrate is that the substrate itself and the core molecule released by hydrolysis, should not be inhibitory to microbial growth (Manafi *et al.*, 1991).

Fluorescein and resorufin are also examples of fluorogenic compounds used in microbiology. Resorufin is a compound which produces a red fluorescence, (excitation wavelength of 570 nm, emission wavelength of 585 nm) and like the coumarin-based substrates, incorporates one moiety for enzyme activity (Haughland, 1996). Fluorescein is a compound which produces a green fluorescence (excitation wavelength of 494 nm, emission wavelength of 518 nm). However, unlike coumarin, substrates based on fluorescein usually incorporate two moieties e.g. fluorescein di-ß-glucoside. Each moiety serves as a substrate for enzyme activity.

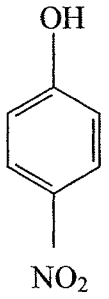
Fluorogenic compounds are best suited for use in liquid assays, such as tube assays and multi-well formats (Manafi *et al.*, 1991). This is due to the extensive diffusion of the water-soluble product. Conversely, these substrates have little use in solid-based media. The reason for this being that the fluorescent product diffuses away from those bacteria expressing the target enzyme, making it difficult to differentiate positive and negative bacterial colonies in mixed cultures.

Chromogenic enzyme substrates

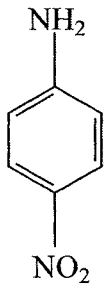
Although it is claimed that the use of a fluorogenic substrate may greatly enhance the detection of enzymatic activity when compared to a chromogenic substrate (Fujiwara and Tsuru, 1978; Coburn *et al.*, 1986), due to their pH dependence, strong diffusion in solid media and the requirement of UV light, the use of these substrates can be limited. Chromogenic enzyme substrates are compounds which act as the substrate for specific enzymes and change colour due to the action of the enzyme. The derivatives of the following chromogenic substrates have since been used most frequently: esters of *ortho-*

nitrophenol (*o*NP) or *para*-nitrophenol (*p*NP) (Le Minor and Ben Hamida, 1962), esters of indoxyl or 5-bromo-4-chloro-3-indoxyl (Bürger, 1967), and peptides of *para*-nitroaniline (*p*NA) (Le Minor and Ben Hamida, 1962). These substrates are colourless, but release of nitrophenol or nitroaniline results in an increase in A_{405} and the appearance of a yellow colour that is most intense at pH 7.9 (Trepeta and Edberg, 1987). Release of indoxyl results in the appearance of a blue colour (Bürger, 1967; Ley *et al.*, 1988). The structures of *p*NP and *p*NA are shown in Figure 3.5.

Figure 3.5: Structures of (i) *para*-nitrophenol and (ii) *para*-nitroaniline



(i) *p*-nitrophenol



(ii) *p*-nitroaniline

These substrates, esters of *o*NP, *p*NP and *p*NA, and their products, are soluble in water and as a result are widely used in liquid assays, such as multi-well assays (Le Minor and Ben Hamida, 1962; Bürger, 1967). However, they are less suitable for agar-based culture as the coloured product diffuses away from positive colonies, making identification difficult.

Figures 3.6 and 3.7 illustrates two such commonly used chromogenic enzyme substrates, *ortho*-nitrophenyl-ß-D-galactopyranoside (ONPG), hydrolysed by ß-

galactosidase, and L-alanyl-*p*-nitroanilide, hydrolysed by alanine aminopeptidase. These figures also illustrate the resulting yellow coloured molecules, *o*NP and *p*NA respectively, subsequent to hydrolysis. The coloured products can be observed visually or detected spectrophotometrically. A number of these substrates have been employed to look at *B. cepacia* complex strains and closely related organisms (Welch *et al.*, 1987, Kämpfer *et al.*, 1988; Wauters *et al.*, 1995; Henry *et al.*, 2001).

Figure 3.6: Hydrolysis of colourless *ortho*-nitrophenyl-ß-D-galactopyranoside (ONPG) to the yellow-coloured *ortho*-nitrophenol

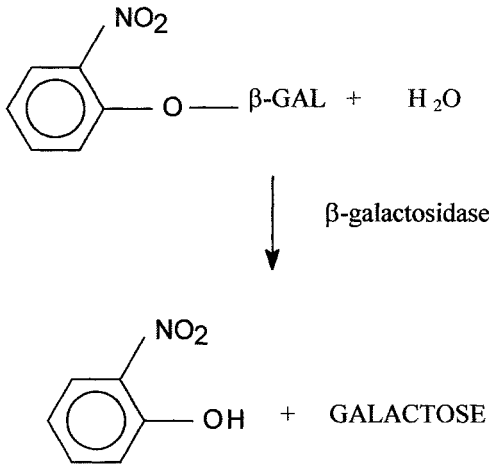
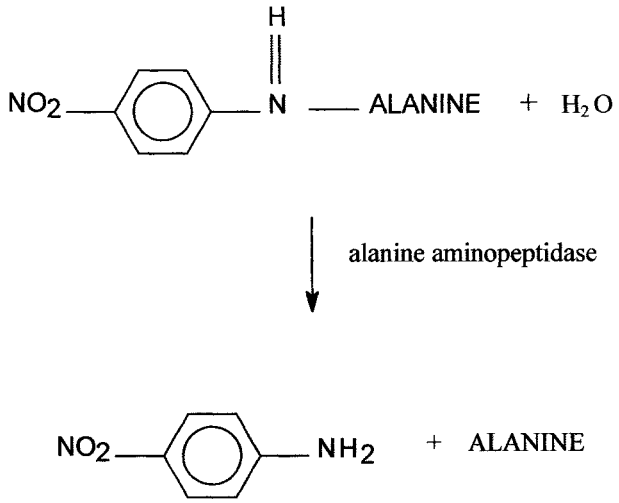


Figure 3.7: Hydrolysis of colourless L-alanyl-*para*-nitroaniline to the yellow-coloured *para*-nitroaniline



Applications of enzyme substrates

Hydrolases are the most commonly sought class of enzymes for differentiating organisms in diagnostic microbiology. These enzymes are commonly found in many bacteria and are very useful to differentiate between closely related species. This class comprises some 30-40 % of all known enzymes and may be divided into various subclasses such as the esterases, glycosidases, phosphatases, proteases/peptidases and sulphatases (Bascomb, 1987). Reactions are usually unidirectional or at least go predominantly to completion, such that the end-product of the reaction is clearly visible (Bascomb, 1987). All of these enzymes are hydrolytic so that, apart from substrate and enzyme, the only other component needed is water. Additionally, in the microbial cell, the enzyme is usually accessible, being extracytoplasmic, or extracellular or non-localized in the cytoplasm i.e. not bound to intracellular structures (James 1994).

Glycosidases

The glycosidases represent a large group of hydrolytic enzymes which have great importance; they are one of the most commonly sought groups of enzymes for bacterial identification (Koneman *et al.*, 1992). Their role in the natural state probably involves the degradation of external carbohydrate sources such as hyaluronic acid, mannans, starches and xylans and the turnover of microbial polysaccharides (James, 1994). Two sub groups of the glycosidases exist, defined by the method of linkage of the sugar unit to the aglycone (the non-carbohydrate moiety), ∝ and ß. Glycosidase substrates are highly useful tools as they exhibit very high selectivity for hydrolysis of their preferred sugars, e.g. ß-galactosidase hydrolyses ß-galactosides but not the enantiomeric α-galactosides or the isomeric ß-glucosides (Haughland, 1996). The hydrolytic activities of the glycosidase enzymes have been extensively studied for the differentiation of Enterobacteriaceae, facilitated by the use of chromogenic or fluorogenic substrates (Le Minor and Ben Hamida, 1962; Perez *et al.*, 1986). Bcc and closely related organisms have been investigated for their production of glycosidases, although little is known of the differences in glycosidase production between the species comprising the Bcc (Welch *et al.*, 1987; Kämpfer and Dott, 1988; Wauters *et al.*, 1995; Henry *et al.*, 2001).

Several glycosidases have been reported as important taxonomic markers. The enzyme ß-galactosidase, for example, catalyses the breakdown of lactose into galactose and glucose, and the detection of this enzyme is used in diagnostic microbiology for the differentiation of *E. coli* and other coliforms from other members of the Enterobacteriaceae (Warren *et al.*, 1978; Manafi *et al.*, 1991). A number of "*B. cepacia*" strains have been found to produce this enzyme (Kämpfer and Dott, 1988). ß-

glucosidase is an enzyme that cleaves di-and oligosaccharides containing ß 1-4 linked glucose, such as seen in esculin and cellobiose (Bürger, 1967; Kilian and Bülow, 1976) and is mainly used for differentiation and identification of group D streptococci (Facklam, 1972), *Listeria monocytogenes* (van Netten *et al.*, 1989) and some Enterobacteriaceae, such as *Klebsiella, Serratia* and *Enterobacter* (Edwards and Ewing, 1986). ß-glucosidase has also been found to be produced by a number of "*B. cepacia*" strains (Kämpfer and Dott, 1988). Both ß-galactosidase and ß-glucosidase were found to be produced by only a proportion of "*B. cepacia*" isolates screened, perhaps indicating differences in enzyme production between the different Bcc species (Kämpfer and Dott, 1988). The enzyme ß-glucuronidase is involved in the decomposition of the intercellular substances of host connective tissue. It catalyses the hydrolysis of a ß-D-glucuronide derivative into an aglycone and glucuronic acid (Manafi *et al.*, 1991) and is used for differentiating between strains of pathogenic *E. coli* O157 and commensal strains of *E. coli* (Dahlén and Linde, 1973; Bascomb, 1987; Manafi *et al.*, 1991), and also in the characterization of streptococci (Röd *et al.*, 1974). The enzyme N-acetyl-ß-D-glucosaminidase has been found to be produced by "*B. cepacia*" strains but not by closely related organisms including *Pandoraea* spp. and *Alcaligenes* spp., and therefore could potentially act as a marker for Bcc strains (Kämpfer and Dott, 1988). Kilian and Bulow (1976) described a number of tests for the rapid detection of glycosidases in Enterobacteriaceae, including α-glucosidase, ß-glucosidase, ß-glucuronidase, ß-xylosidase and α-fucosidase using *p*NP-linked substrates.

Peptidases

Peptidases are involved in the process by which proteins and peptides are degraded into amino acids (Bascomb 1987). More specifically they are involved in the utilization of peptides as sources of amino acids for protein synthesis or as the source of carbon and nitrogen. Peptidases fall into three groups, aminopeptidases, carboxypeptidases and endopeptidases.

a) Aminopeptidases

Aminopeptidases generally have broad specificity and occur in several forms. They are widely distributed and have been found in many tissues or cells, on cell surfaces, and in soluble or secreted forms in plants and animals (Taylor, 1983; Stirling, 1989; Ahmad and Ward, 1990). Aminopeptidases are responsible for catalyzing the stepwise cleavage of a single amino acid from the amino terminus of a peptide. The structure, function and proposed mechanism of action of aminopeptidases have been reviewed by Taylor (1993a, 1993b). Among the suggested functions of these enzymes are terminal degradation of proteins, hormone level regulation, and cell-cycle control. They constitute a substantial proportion of the enzyme protein in various cells (Watt and Yip, 1989). Aminopeptidases are established as taxonomic markers in diagnostic microbiology (Muftic, 1967; Westley *et al.,* 1967; Peterson and Hsu, 1978; Bascomb, 1987). Substrates are formed by the linkage of an amino acid or small peptide, via a carboxyl group, to a fluorogenic or chromogenic amine e.g. 7AMC or *p*NA. In earlier studies, derivatives of naphthylamine were used and enzyme activity was detected

fluorometrically or by reaction with diazonium dyes (Bascomb 1987). However, the known carcinogenic nature of naphthylamide substrates made it desirable to find alternative aminopeptidase substrates (Godsey *et al.,* 1981). Several aminopeptidases have been identified as important taxonomic markers including derivatives of alanine, pyroglutamic acid, proline and *gamma*-glutamine.

One of the best studied aminopeptidase enzymes is L-alanine aminopeptidase. Alanine aminopeptidase is a glycoprotein found in many tissues and biological fluids. In clinical chemistry, determination of serum AAP activity is used for detecting or confirming biliary obstruction from either intra or extra hepatic disorders (Sanderink *et al.*, 1988). It appears to be produced universally by Gram negative bacteria (Murgier *et al.*, 1976; Manafi and Kneifel, 1990), including "*B. cepacia*" (Kämpfer and Dott, 1988) and so may be used to differentiate between Gram positive and Gram negative species, with more activity occurring in Gram-negative species (Cerny 1976, 1978; Manafi and Kniefel, 1990). A number of substrates have been evaluated for this assay, including L-alanine-AMC (Ramsey *et al.*, 1980). Pyrrolidonyl (PYR) substrates, which detect pyrrolidonyl aminopeptidase are primarily used for the differentiation of group A streptococci and enterococci, which are PYR arylamidase positive, from other species (Facklam *et al.*, 1982, 1984; You and Facklam, 1986). Substrates used include PYR-*p*NA and PYR-AMC (Fujiwara and Tsuru 1978). Proline aminopeptidase has been suggested as a useful enzyme for the differentiation of *Serratia marcescens* from other Enterobacteriaceae and one substrate that has been used for the detection of this enzyme assay is prolyl-ß-naphthylamide (Godsey *et al.*, 1981). *Gamma*-glutamyl ß-naphthylamide is used to distinguish between *Neisseria meningitidis* and *Neisseria gonorrhoeae* (D'Amato *et al.,* 1978). A test for the detection of benzyl-arginine

arylamidase is used in commercial systems for the differentiation of *Pseudomonas* sp. and *Burkholderia* sp. (Laffineur *et al.*, 2002).

The production of aminopeptidases has been investigated to a limited degree in "*B. cepacia*" isolates and these strains have been found to produce various aminopeptidases other than alanine aminopeptidase including leucine aminopeptidase and lysine aminopeptidase (Kämpfer and Dott, 1988; Wauters *et al.*, 1995). Only a proportion of "*B. cepacia*" isolates screened have been found to produce arginine aminopeptidase, glycine aminopeptidase and proline aminopeptidase, again perhaps indicating a difference in the production of these enzymes from the different species comprising the Bcc (Kämpfer and Dott, 1988).

b) Carboxypeptidases

There are few substrates available for detection of carboxypeptidases. This is mainly due to the inherent difficulty in generating substrates that produce a bright colour or fluorescence after enzyme activity. Those substrates which are available are formed by the linkage of an amino acid or small peptide, via an amino group, to a non-metabolic moiety. An example of such a moiety is benzoic acid. Currently, the main application of a carboxypeptidase substrate is in the hippurate test. In this test, the substrate, hippuric acid, (benzoyl-glycine; benzoylaminoacetic acid) is hydrolysed by a glycyl carboxypeptidase to release benzoic acid and glycine, the latter being detected by addition of ninhydrin to yield a deep purple coloration (Ellis and Walker, 1942; Hwang and Ederer 1975; Perry *et al.*, 1998). The hippurate test is used to distinguish between

strains of haemolytic streptococci group B (hippurate positive) and haemolytic streptococci group A (hippurate negative). It can also be used for the detection of *L. monocytogenes*, and for differentiation between *Campylobacter coli and C. jejuni* (On and Holmes, 1992). N-benzoyl-L-glutamic acid can be used to differentiate between *Pseudomonas fluorescens* and *P. aeruginosa*; and benzoyl-L-alanine can be used to distinguish between *Enterobacter aerogenes* and *Enterobacter cloacae* (Perry *et al.*, 1998). Perry *et al.* (1998) looked at the detection of carboxypeptidases as taxonomic markers for Gram negative bacteria, including "*B. cepacia*". All "*B. cepacia*" strains produced glycine carboxypeptidase. A proportion of the "*B. cepacia*" strains produced alanine carboxypeptidase, methionine carboxypeptidase and phenylalanine carboxypeptidase.

c) Endopeptidases

Endopeptidase enzymes act on specific, target peptide bonds within amino acid chains. Synthetic aglycone derivatised tri- and tetrapeptides are sensitive and specific tools for demonstrating bacterial protease activity. The aglycone (e.g. *p*NA or 7AMC) is attached to the carboxy-terminal end of the peptide chain via an amide bond. Upon hydrolysis, the bond between the peptide and aglycone is broken, resulting in a bright fluorescence or colour due to the release of the fluorogenic or chromogenic aglycone (Berdal and Olsvick, 1983).

An example of the application of endopeptidase activity detection in diagnostic microbiology is in the detection of staphylocoagulase produced by *Staphylococcus aureus*. Several chromogenic and fluorogenic substrates have been developed for this

assay, such as TBOC-valyl-prolyl-arginyl-7-amido-4-methylcoumarin. Other examples include the substrates glutarylphenylalanine-*p*NA and glutarylphenylalanine-AMC which are available for the detection of chymotrypsin (Zimmerman *et al.*, 1976). Benzoyl-L-arginyl-7AMC and benzoyl-L-arginyl-*p*NA are substrates for trypsin and can be used for the rapid detection of *Vibrio parahaemolyticus* in food (Miyamoto *et al.*, 1990). Succinyl-$(Ala)_3$-*p*-nitroaniline has been widely used as a synthetic substrate for assaying pancreatic elastase 1 (Kuwada, 1984). Chromogenic peptide substrates are established in applications for the quantitation of proteolytic enzyme activities in biological samples (Witt, 1977, Skully and Kakkar, 1979). For example, the chromogenic substrates H-D-Valyl-Leucyl-Lysyl-*p*NA and Bz-Phenyl-Valyl-Arginyl-*p*NA have been used to measure rates of proteolysis of plasmin (Friberger *et al.*, 1979) and thrombin (Svendsen *et al.*, 1972), respectively, and the fluorogenic substrate MeO-succinyl-Alanyl-Alanyl-Prolyl-Valyl-AMC is capable of monitoring the amidolytic activity of elastase (Castillo *et al.*, 1979). The substrate succinyl-phenylalanyl-*p*NA has been used to screen for the endopeptidase chymotrypsin activity in "*B. cepacia*" strains and closely related organisms. No such organisms were found to produce this enzyme (Kämpfer and Dott, 1988). The endopeptidases 5`-nucleotide phosphodiesterase and phospholipase C have been found to be produced by a number of "*B. cepacia*" isolates but not by closely related organisms such as *Pandoraea* spp. (Kämpfer and Dott, 1988; Wauters *et al.*, 1995).

Esterases

Esterase enzymes are less commonly sought than glycosidase enzymes for the differentiation of microbial species. These enzymes hydrolyse molecules with shorter

chain organic acids (Manafi *et al.*, 1991). There are several substrates currently available for the detection of esterase activity. These mainly include derivatives of MU, fluorescein, and indoxyl (Guilbault and Kramer 1965; Popovic-Uroic *et al.*, 1990). Use of butyryl, hexanoyl, heptanoyl, nonanoyl, palmityl and oleyl esters of MU has been described (Kloosterziel *et al.*, 1976; Manafi *et al.*, 1991; Speeleveld *et al.*, 1994). Some examples of esterases used in diagnostic microbiology include acetate esterase, butyrate esterase and octanoate esterase. Acetate esterase can be used as a rapid and selective differential test to distinguish between a limited number of *Campylobacter* sp. and related genera, including *Helicobacter* and *Wolinella* (Popovic-Uroic *et al.*, 1990). Butyrate esterase has been primarily used to distinguish *Moraxella catarrhalis* from other oxidase-positive, Gram-negative cocci, including members of family Neisseriaceae (Dealler *et al.*, 1989; Manafi *et al.*, 1991; Speeleveld *et al.*, 1994). All strains of *Salmonella* sp. are positive for octanoate esterase, making this a useful assay for the identification of *Salmonella* sp. from clinical and food samples.

Miscellaneous enzymes

Other enzymes, production of which have been screened for in "*B. cepacia*" strains and closely related organisms include alkaline phosphatase, benzyl-arginine arylamidase and pyrrolidonyl arylamidase (Laffineur *et al.*, 2002). Some of these enzymes seem to have potential use as markers for Bcc strains. For example, pyrrolidonyl arylamidase can differentiate between Bcc strains, which produce the enzyme, and *B. gladioli* and *R. pickettii*, which do not. Sulphatase was found to be produced by number of Bcc isolates, but not by all of those screened (Kämpfer and Dott, 1988; Laffineur *et al.*, 2002).

Carbohydrate oxidation

Detection of acid production from the oxidation of carbohydrates can be a very useful tool in diagnostic microbiology. It is a commonly used method for simple, user-friendly, inexpensive differentiation between organisms. The ability to metabolize glucose with the consequence of acid generation is a characteristic of most pathogenic bacteria. Oxidation of glucose is an aerobic process utilized by strict aerobes in which the aldehyde group of glucose is oxidized to form gluconic acid. This is then further oxidized to 2-ketogluconic acid which is eventually degraded to form pyruvic acid (Hugh and Leifson 1953). Almost all strains of staphylococci, streptococci, enterococci and Enterobacteriaceae readily ferment glucose to produce acidic products (MacFaddin, 2000). Other less common pathogens such as *Neisseria* spp. and *Acinetobacter baumannii* and Bcc species are able to generate acid from glucose via oxidation (Janda *et al.*, 1984, Kämpfer *et al.*, 1993).

Carbohydrate fermentation tests, as well as oxidation tests, are commonly adopted in diagnostic microbiology to differentiate between organisms. For example, the API 20E strip (bioMérieux, La Balme les Grottes, France) is an identification system for Enterobacteriaceae which detects fermentation and oxidation of various carbohydrates such as D-mannitol, inositol, D-sorbitol and D-melibiose. However, for non-fermentative Gram negative bacilli, such as members of the Bcc and closely related organisms, such tests are of limited use. In another example, the API 20 NE strip (bioMérieux, La Balme les Grottes, France), which is used in the identification of Bcc strains, contains only a glucose fermentation test, for which all Bcc strains are negative and therefore no differentiation is achieved between such organisms. Bcc strains,

however, are nutritionally versatile and are capable of assimilating a large variety of carbon sources (Yabuuchi *et al.*, 2000; Vermis *et al.*, 2003) including compounds such as azelaic acid and tryptamine (Burbage and Sasser, 1982). Heterogeneity in carbon source utilisation has been demonstrated within the Bcc, but also within the individual species belonging to this complex (Vermis *et al.*, 2003). However, it may be possible that differentiation between members of the Bcc could be achieved by investigating differences in carbohydrate oxidation, rather than assimilation. Few studies have been performed on the carbohydrate oxidative profiles of the Bcc organisms and some closely related species such as *Pandoraea* spp and those which have been performed often date before the description of all nine genomovars (Welch *et al.*, 1987; Lewin *et al.*, 1993; Wauters *et al.*, 1995; Pitt *et al.*, 1996), or ignore the recent taxonomic additions (Bonacorsi *et al.*, 1999; Bevivino *et al.*, 2002; Laffineur *et al.*, 2002). However, it has been suggested that sucrose and adonitol oxidation may be of some use in differentiation between Bcc strains (Henry *et al.*, 2001).

Because of the diversity of end products of carbohydrate metabolism, it is impossible to target a specific product as a universal indicator of oxidation or fermentation. It is therefore necessary to measure pH decrease as a universal means of determining oxidation of the carbohydrate tested.

In an effort to improve the speed and accuracy of the identification of Bcc strains, this project looks at the application of enzymatic profiling of microorganisms by using fluorogenic and chromogenic substrates, as well as carbohydrate oxidation profiles.

Experimental objectives

1 – To evaluate the differential capability of a total of 85 enzyme substrates, specifically 51 fluorogenic and 34 chromogenic substrates (including 13 endopeptidase, 7 benzoyl amino acid, and 14 miscellaneous substrates) for the identification of Bcc strains and closely related organisms using a collection of 183 organisms consisting of 33 Bcc strains, 75 *P. aeruginosa* strains, and various other species closely related to the Bcc

2 – To further investigate any discriminatory, reproducible enzyme substrates by screening these with an extended panel of 310 organisms, consisting mainly of Bcc strains to specifically detect any differentiation between the species comprising the Bcc.

3 – To assess the diagnostic potential of 49 carbohydrate oxidative tests in differentiating specifically between the Bcc species using a sample of 30 control strains, followed by an extended panel of 282 strains.

Materials

Bacterial strains

The bacterial strains and isolates screened in this study can be divided into two collections that were screened separately.

Initial screen collection (Collection A)

Of the 183 strains used in this initial screen, 20 *P. aeruginosa* isolates were cultured from pre-operative bronchoalveolar lavages (BAL) of CF lung transplant patients in the Microbiology Department, Freeman Hospital, Newcastle upon Tyne, (Appendix 3.1) and were identified using Analytical Profile Index (API) 20NE strips and associated software (bioMérieux, La Balme-les-Grottes, France) and C390 sensitivity tests (Biosynth AG, UK). Fifty two *P. aeruginosa* strains were obtained from the British Society for Antimicrobial Chemotherapy (BSAC) (Andrews *et al.*, 2002). Seventy five organisms were obtained from the Belgian Coordinated Collections of Microorganisms (BCCM), 30 of which were a documented reference panel of Bcc organisms including genomovars I - V (Mahenthiralingam *et al.*, 2000b), the remaining 45 strains being organisms closely related to Bcc; *Burkholderia sp.*: *B. ambifaria*, LMG 11351, *B. andropogonis*, LMG 1279 and LMG 2126, *B. caryphylli*, LMG 2155 and LMG 2156, *B. cepacia* (GVI), LMG 18941 and LMG 18942, *B. gladioli*, LMG 11626 and LMG 18113, *B. gladioli pv. alliicola*, LMG 2121 and LMG 6877, *B. gladioli pv. gladioli*, LMG 2216 and LMG 6880, *B. glumae*, LMG 1277 and LMG 2196, *B. phenazinium*, LMG 2247 and LMG 6868; *Pandoraea sp.*: *P. apista*, LMG 16408, *P. norimberensis*,

LMG 13019 and LMG 16603, *P. pnomenusa*, LMG 18087 and LMG 18817, *P. pulmonicola*, LMG 18107, *P. sputorum*, LMG 18100 and LMG 18819; *Ralstonia sp.*: *R. basilensis*, LMG 18990 and LMG 19286, *R. campinensis*, LMG 19282 and LMG 19283, *R. eutropha*, LMG 1190 and LMG 1194, *R. gilardii*, LMG 3399 and LMG 3400, *R. mannitolilytica*, LMG 19090, *R. metallidurans*, LMG 1195 and LMG 19290, *R. paucula*, LMG 3244 and LMG 3245, *R. pickettii*, LMG 5942 and LMG 6871, *R. solanacearum*, LMG 2291 and LMG 2293, *R. taiwanensis*, LMG 19425, *Stenotrophomonas maltophilia*, LMG 957 and LMG 958. Thirty seven strains of non-fermenting organisms were obtained from the NCTC, ATCC and NCIMB; *Acinetobacter sp.*: *A. baumannii*, ATCC 19606, *A. calcoaceticus*, NCTC 7844, *A. haemolyticus*, NCTC 12155, *A. johnsonii*, NCTC 10308, *A. lwoffi*, NCTC 5866, NCTC 5867, NCIMB 12456; *Brevundimonas diminuta*, ATCC 11568, *Brevundimonas vesicularis*, ATCC 11426; *Cryseobacterium meningosepticum*, ATCC 13253, *Moraxella nonliquefaciens*, NCTC 10464, *Moraxella osloensis*, NCTC 10465, *Moraxella urethralis*, NCTC 11010, *Oligella urethralis*, NCTC 11999; *Pseudomonas sp.*: *P. acidovorans*, NCTC 10683, *P. aeruginosa*, NCTC 6749, NCTC 10332 and NCTC 10662, *P. alcaligenes*, NCTC 10367, *P. pseudoalcaligenes*, NCTC 10860, *P. diminuta*, NCTC 8545, *P. fluorescens*, NCTC 10754, *P. fluorescens*, NCTC 10392, *P. fluorescens*, NCTC 3756, NCTC 10038, NCTC 10688, NCTC 9428, *P. fragi*, NCIMB 8987, *P. maltophilia*, NCTC 10257, *P. paucimobilis*, NCTC 11030, *P. putida*, NCTC 10936, *P. stutzeri*, NCTC 12262 and NCTC 10475, *P. vesiculare*, NCTC 10900; *Ralstonia pickettii*, NCTC 11149 and NCTC 11149, *Sphingobacterium spiritivorum*, ATCC 33861. Two control strains were included which were *E. coli*, NCTC 10418, and *Enterobacter cloacae*, NCTC11936.

Extended screen collection (Collection B)

The 310 Bcc isolates and closely related organisms comprising the extended panel of strains were from a collection of isolates previously identified as being members of the Bcc in various international laboratories, and referred for confirmation to the University of British Columbia, Vancouver. A total of 282 isolates were members of the Bcc, and 28 organisms belonged to phenotypically similar species frequently mis-identified as belonging to the Bcc. Isolates were selected from a wider collection to represent a variety of geographical and epidemiological groups, and include clinical isolates cultured from both CF and non-CF patients, and environmental strains. Isolates were identified as described by Henry *et al.* (1997). Table 3.1 shows the composition of the collection.

Table 3.1: Strains comprising the extended panel of isolates (Collection B)

Strain	No. of isolates
Acinetobacter sp.	1
Alcaligenes sp.	12
B. cepacia	36
B. multivorans	48
B. cenocepacia III-A	50
B. cenocepacia III-B	30
B. stabilis	32
B. vietnamiensis	42
B. dolosa	18
B. ambifaria	11
B. anthina	13
B. pyrrocinia	2
Pandoraea sp.	5
Ralstonia pickettii	5
Stenotrophomonas maltophilia	5
Total	**310**

Growth media

Yeast extract, tryptone and proteose peptone were obtained from Oxoid Ltd (Basingstoke, UK). Columbia agar was also obtained from Oxoid, prepared according to manufacturers instructions and supplemented with 5 % defibrinated horse blood from TCS Biosciences Ltd. (Buckingham, UK). Sodium chloride and phenol red were obtained from BDH (Poole, UK). Ferric ammonium citrate (FAC), potassium aluminium sulphate (PAS) and Tween 20 were obtained from Sigma-Aldrich Company Ltd. (Poole, UK).

Enzyme substrates

Glycosidase substrates; 4-methylumbelliferyl (MU) derivatives of ß-D-glucuronide, α-D-glucuronide, α-D-mannopyranoside, ß-D-ribofuranoside, ß-D-xylopyranoside, were obtained from Glycosynth, Warrington, UK; MU derivatives of: α-L-arabinopyranoside, ß-D-cellobioside, ß-D-fucoside, α-D-galactopyranoside, ß-D-galactopyranoside, α-D-glucopyranoside, ß-D-glucopyranoside, N-acetyl-ß-D-glucosaminide, ß-D-mannopyranoside were obtained from Sigma-Aldrich Company Ltd. (Poole, UK).

Aminopeptidase substrates; 7-amido-4-methylcoumarin (7AMC) derivatives of ß-alanine, L-alanine, L-arginine, Benzyloxycarbonyl-arginine, L-aspartic acid, L-asparginine, L-glutamine, L-glutamic acid, glycine, glycyl-L-proline, L-histidine, L-isoleucine, L-leucine, L-lysine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamine, L-threonine, L-tyrosine, L-valine were obtained from Bachem (Saffron Walden, UK).

Esterase substrates; MU derivatives of nonanoate and lignocerate were obtained from Glycosynth (Warrington, UK); MU derivative of laurate was obtained from NBS Biologicals, Cambridgeshire, UK, MU derivatives of acetate, propionate, butyrate, heptanoate, palmitate, elaidate, stearate and oleate were obtained from Sigma-Aldrich Company Ltd. (Poole, UK).

Carboxypeptidase substrates; benzoyl derivatives of alanine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine were obtained from Sigma-Aldrich Company Ltd. (Poole, UK).

Endopeptidase substrates; the following *para*-nitroanilide (*p*NA) derivatives were obtained from Bachem (Saffron Walden, UK): Ac-alanyl-alanyl-alanine-*p*NA, H-glutamyl-glycyl-arginine-*p*NA, Succinyl-alanyl-alanyl-prolyl-phenylalanine-*p*NA, Succinyl-phenylalanine-leucyl-phenylalanine-*p*NA, H-alanyl-alanyl-phenylalanine-*p*NA, H-cysteine(benzoyl)-*p*NA, Ac-phenylalanine-*p*NA, H-glycyl-phenylalanine-*p*NA, Succinyl-alanyl-alanyl-alanine-*p*NA, Ac-alanyl-alanyl-prolyl-alanine-*p*NA, Succinyl-alanyl-leucyl-prolyl-phenylalanine-*p*NA, Bz-DL-arginyl-*p*NA and H-glutamyl-alanyl-glycine-*p*NA

Chromogenic substrates for application in media; 5-bromo-4-chloro-3-indolyl-N-acetyl-ß-D-glucosaminide (XNAG) and magenta caprylate were obtained from Glycosynth, (Warrington, UK). The following substrates were all kindly synthesised and supplied by Dr. A L. James, Northumbria University (Newcastle, UK): alizarin glucoside, ß-alanyl aminophenyl acridine (ß-ala-APA) and L-alanyl-cresyl violet oxazone (ala-CVO).

Miscellaneous substrates; MU derivatives of phosphate and sulfate were obtained from Sigma-Aldrich Company Ltd., Poole, UK; *para*-nitrophenol derivatives of caprate, ß-L-arabinopyranoside, p`-guanidinobenzoate, caprylate, myristate, L-rhamnopyranoside, phosphorylcholine, phenyl-phosphonate, α-L-fucoside, ß-D-lactopyranoside, α-D-maltoside, ß-D-maltoside, α-D-xylopyranoside, valerate were obtained from Sigma-Aldrich Company Ltd. (Poole, UK).

Carbohydrates

Adonitol, amygdaline, arbutine, cellobiose, D-Fructose, maltose, melibiose, D-raffinose, ribose, salicine, sorbitol and trehalose were obtained from Sigma-Aldrich Company Ltd. (Poole, UK).

Other chemicals and solvents

Phosphate buffered saline, ninhydrin, dimethyl sulphoxide (DMSO), acetone and ethanol were obtained from Sigma-Aldrich Company Ltd. (Poole, UK). Sodium hydroxide and hydrochloric acid were obtained from VWR International (Leicester, UK).

Equipment

All substrates were prepared using a Satorius 2434 electronic balance; accurate to 0.1 mg (Satorius Ltd, Epsom, UK). The pH of the phosphate buffer used for substrate preparation was checked using a pH meter (Hanna Instruments Ltd, Leighton Buzzard,

UK). Acrodisc filters, 0.2 μm, (Gelman Sciences, Ann Arbour, USA) and 10 ml sterile syringes (Terumo Europe N.V., Leuven, Belgium) were used to filter sterilise substrate solutions. Small volumes were dispensed using calibrated Gilsen semi-automatic pipettes (P20, P200 and P1000) with sterile disposable tips (Gilsen Medical Electronics, Villiers-le-Bel, France). Large volumes were dispensed using sterile disposable 10 ml pipettes (L.I.P. Ltd, Shipley, UK). Plastic consumables, including 3 ml plastic graduated pastettes, 25 ml universals and sterile petri dishes, were obtained from Bibby Sterilin Ltd. (Aberbargoed, UK). All organisms were prepared to a specific suspension density using a Densimat (bioMérieux, Marcy L`Etoile, France). Microtitre trays (Bibby Sterilin Ltd., Aberbargoed, UK) with 96 flat-bottomed wells were used. All microtitre trays were incubated in a LEEC 30 ^{O}C incubator (Laboratory and Electrical Engineering Company, Nottingham, UK). An Anthos 2001 spectophotometric plate reader (Labtech International Limited, Uckfield, UK), described below, was used to measure the change in absorbance. A Labtech Biolite F1 fluorescence microtitre plate reader (Labtech International Limited, Uckfield, UK), described below, was used to measure fluorescence in enzyme assays involving fluorogenic substrates. A MSE Sanyo Centaur 2 centrifuge (Fisons Scientific, Sussex, UK) was used to spin the microtitre trays for the evaluation of carboxypeptidase substrates.

Anthos 2001 spectrophotometric microtitre plate reader

(Labtech International Limited, Uckfield, UK)

This is a microprocessor-controlled photometer utilising nine halogen lamps which emit light beams through a diaphragm, an infra-red absorbance glass, a system of lenses and a narrow-band di-electric interference filter. The halogen lamps are selected with the

same spectrum of emission to guarantee identical measurements in each of the nine channels. Eight photo-diodes detect the transmitted light and the ninth diode is used for regulation of constant light energy. Each measurement channel is calibrated prior to every measurement and the absorbance values were calculated by microprocessor. An absorption wavelength of 405nm was used for measuring absorbance of *p*-NP and *p*-NA substrates, and 540nm for benzoyl substrates. A total of 96 readings were accomplished in less than three seconds. Data processing was performed using a combination of Arcom for Windows ® software (MR Electronics) and Microsoft Excel ® 5.0 using a standard personal computer.

Labtech Biolite F1 fluorescence microtitre plate reader

(Labtech International Limited, Uckfield, UK)

This is a microprocessor controlled fluorometer utilising a single optically-stabilised M32-type Tungsten-Halogen lamp with a single IP28 photomultiplier tube for detection. Up to six rotating filters are housed in the instrument each having a band-width of between 20 and 40nm. In all cases, excitation and emission filters at 365/440 nm were used. The use of a single light source and a single detection system maximised reproducibility but resulted in a prolonged reading time of approximately 32 seconds for 96 wells. Data processing was performed using a combination of Biolite software (Astroscan Ltd) and Microsoft Excel ® 5.0 using a standard personal computer.

Methods

Bacterial strain preparation

All strains were stored on lenticules at -20 ^{O}C as previously described (Codd *et al.*, 1998; Lightfoot *et al.*, 2001). When required, strains were cultured as described in Chapter 2.

The following protocols apply to both organism collections A and B, unless otherwise indicated.

Evaluation of fluorogenic and chromogenic substrates for differentiation of Bcc and closely related species.

A total of 14 glycosidase substrates, 21 aminopeptidase substrates, 11 esterase substrates, 1 phosphatase substrate, 1 sulphatase substrate, 16 endopeptidase and 14 miscellaneous chromogenic substrates were evaluated. The amount of 0.035mmoles of each substrate were weighed and dissolved in 12 ml phosphate buffer. For example for 4-MU-ß-D-galactoside (RMM 338.32), 12 mg of substrate were dissolved in 12 ml of phosphate buffer pH7.4 (23 g/l dipotassium hydrogen phosphate; 5.92 g/l potassium dihydrogen phosphate) to produce a solution of 1 mg/ml or 2.96 mmol/l. Esterase substrates were first dissolved in 400 µl dimethylsulfoxide (DMSO), then added to 11.6 ml phosphate buffer. Dissolution was aided by gentle heating in a water bath at lowest temperature required up to a maximum of 80 ^{O}C (esterase substrates were not heated). Bacterial suspensions of all organisms with turbidity equivalent to 1 McFarland (3 x 10^{8}

cfu/ml) were prepared in sterile distilled water. Each bacterial suspension was added to microtitre trays in 50 µl aliquots followed by the addition of an equal volume of substrate. This was intended to produce an inoculum of approximately $1.5x10^7$ cfu. These tests were performed in batches of eight substrates. Trays containing fluorogenic substrates were read using a Labtech Biolite F1 fluorescence microtitre plate reader set at 365 nm excitation wavelength and 440 nm emission wavelengths (time zero). The trays were incubated at 30 ^{O}C for 18 hrs in aerobic conditions then read once more on the fluorometer. The time zero values and the averaged negative control values were subtracted from these results. A value was considered positive if the resulting figure was above 1000. Trays containing chromogenic substrates had the absorbance determined at 405 nm immediately using an Anthos 2001 spectrophotometric microtitre plate reader (time zero). The trays were incubated at 30 ^{O}C for 72 hrs in aerobic conditions. Trays were read by spectrophotometer after 24, 48 and 72 hrs. The time zero values and the averaged negative control values were subtracted from these results. Visual recordings were made of +/-, +, ++ or +++, according to colour intensity. The result was considered positive if the yellow colouration was recorded as "+" after 48 hrs. Any substrates which looked to be of discriminative value were screened in duplicate.

Evaluation of carboxypeptidase substrates for differentiation of BCC and closely related species.

This study was performed on organism collection A only

a) Investigation into the optimal technique for evaluation of carboxypeptidase substrates

A centrifugation technique has previously been used to evaluate carboxypeptidase substrates (Perry *et al.*, 1998) but is laborious and non-user friendly. Therefore a non-centrifugation technique was evaluated in comparison using the BCCM panel of 30 Bcc organisms to see if this one would be more suitable for the large-scale testing of the initial screen collection of organisms. A total of 7 benzoyl-L-amino acids were evaluated.

Each substrate was weighed out to achieve a 2 % solution (i.e. 100 mg), apart from N-benzoyl-DL-leucine and N-benzoyl-DL-methionine, for which 4 % solutions were prepared (i.e. 200 mg). Each substrate was prepared in 4.5 ml sterile distilled water, with addition of 1 mol/l sodium hydroxide to aid dissolution. The pH was adjusted to 7.4 using 1 mol/l hydrochloric acid. The final volume was adjusted to 5 ml with sterile distilled water and pH re-tested. These solutions were then heated in a water bath for 5 minutes at 100 ^{O}C. Bacterial suspensions of the organisms were prepared to a turbidity equivalent to 7 McFarland (2.1 x 10^9 cfu/ml) in 6ml sterile distilled water. Each bacterial suspension was aseptically dispensed in 100 µl volumes into wells of sterile flat bottomed microtitre trays, followed by the addition of an equal volume of substrate.

Trays were incubated at 30 ^{O}C for 18 hrs in aerobic conditions. These were prepared in duplicate. One set of trays were centrifuged at 3000 g for 10 minutes and 100 µl of the supernatant fluid was transferred to fresh microtitre wells. From the second set of trays, aliquots of 100 µl of the samples from the upper half of each well were transferred to fresh microtitre wells, without centrifugation. An aliquot of 50 µl of ninhydrin solution (350 mg in 10 ml of a 1:1 ethanol/acetone mixture) was added to all wells, from both sets of trays. Absorbance readings were determined at 540 nm immediately (time zero). The trays were incubated at 37 ^{O}C for 30 minutes, and absorbance readings repeated at 540 nm, the intensity of the purple colouration in each well was also noted as +/-, +, ++ or +++. A result was considered positive if purple colouration of at least +, ++ or +++ was observed. All tests were performed in duplicate.

b) screening of collection using non-centrifuge method

The methodology is as described in part a) except the substrates were prepared in 10 x quantity (i.e. 1 g or 2 g in 50 ml to make 2 % and 4 % solutions respectively). The 183 test bacterial suspensions and the two control organisms were aseptically dispensed in 100 µl volumes into 185 wells of two sterile flat bottomed microtitre trays. The remaining seven wells were filled with 100 µl substrate and 100 µl of sterile distilled water, as sterility controls.

Application of non-diffusible chromogenic substrates into Columbia agar based media.

Six different media were prepared containing non-diffusible chromogenic substrates. Each was prepared in a 200 ml Duran bottles. Into each Duran bottle was weighed 4 g Columbia agar, the different media types were then prepared as follows:

1) XNAG – 20 mg substrate and 100 ml sterile distilled water were added to Columbia agar and subsequently boiled by microwave for approximately 3 minutes.

2) Magenta caprylate – 100 ml sterile distilled water was added to Columbia agar and subsequently autoclaved at 121 ^{O}C for 15 minutes and then cooled to 50 ^{O}C.

- 50 mg substrate was dissolved in 0.5 ml DMSO followed by addition of 0.75 ml Tween 20. This solution was then added to the cooled Columbia agar (50 ^{O}C).

3) Alizarin glucoside with ferric ammonium citrate (FAC) –10 mg substrate, 50 mg FAC and 100 ml sterile distilled water were added to Columbia agar and subsequently autoclaved at 121^{O}C for 15 minutes.

4) Alizarin glucoside with potassium aluminium sulphate (PAS) – 10 mg substrate, 50 mg PAS and 100 ml sterile distilled water were added to Columbia agar and subsequently autoclaved at 121 ^{O}C for 15 minutes.

5) ß-ala-APA – 30 mg substrate and 100 ml sterile distilled water were added to Columbia agar and subsequently autoclaved at 121 ^{O}C for 15 minutes.

6) Ala-CVO – 100 ml sterile distilled water was added to Columbia agar and subsequently autoclaved at 121 ^{O}C for 15 minutes, then cooled to 50 ^{O}C

- 5 mg substrate dissolved in 1 ml DMSO was added to the cooled Columbia agar

The media were poured in 20 ml amounts per plate. A10 µl loop was used to inoculate one plate of every media type with a 0.5 McFarland suspension of the following Bcc strains: *B. cepacia,* LMG 1222, *B. cepacia,* LMG 2161, *B. multivorans,* LMG 16656, *B. stabilis,* LMG 14086, *B. stabilis,* LMG 14294 and *P. apista,* LMG 16408.

Evaluation of oxidative tests for differentiation of Bcc species using 50CH strips (bioMérieux).

50CH strips (bioMérieux, France) were used to screen for oxidation of 49 carbohydrates. These strips consisted of 50 micro-tubes each containing an anaerobic zone, for the study of fermentation and an aerobic zone, for the study of oxidation or assimilation. The strips were set up on the 30 BCCM reference panel of Bcc according to manufacturers instructions. One strip was also inoculated with medium only, included as a negative control. Bacterial suspensions were prepared with turbidity equivalent to 0.5 McFarland (1.5×10^8 cfu/ml) using a densitometer in an ampoule of API 50 CHB/E Medium containing phenol red. The strips were incubated at 30 ^{O}C for 48 hrs in aerobic conditions. Two readings of the results were performed, one after 24 hrs and again after 48 hrs. The strips were read in a semi-quantitive way: 0 given to negative reaction (red) and 5 to positive reactions of maximum intensity (yellow). Values of 1, 2, 3 or 4 were assigned to intermediate reactions. A value of 3, 4 and 5 was considered positive as stated in manufacturers instructions.

Evaluation of oxidative tests for differentiation of Bcc and closely related species reproducing 50CH strips (bioMérieux) in microtitre format

Numerous pilot studies were performed (data not included) to evaluate various liquid media constituents and sugar concentrations, based on the 50 CHB/E Medium, and bacterial inoculum sizes, to determine optimum medium and inoculum size which could reproduce the reactions observed in the 50CH strips, in an inexpensive microtitre format suitable for large-scale screening. The medium and bacterial inoculum described in these experiments were those found to be the most effective in distinguishing between positive and negative sugar oxidation reactions.

a) pilot study

A selection of Bcc strains were chosen from the BCCM collection of 30 organisms, to include a representative of each species, (LMG 1222, LMG 2161, LMG 16654, LMG 16659, LMG 18821, LMG 18826, LMG 18832, LMG 18863, LMG 13010, LMG 17588, LMG 18824, LMG 18825, LMG 18888, LMG 18836). Once cultured on Columbia blood agar, a suspension of each organism was prepared in water at a concentration of McFarland 5 (1.5×10^9 cfu/ml).

The medium was prepared in 100 ml volumes as follows:

A 1g amount of proteose peptone, 50 mg of yeast extract, 0.5 g of sodium chloride and 0.1 g tryptone were placed in a glass medical flat. Using a graduated pipette 1.5 ml of 2 % phenol red was added to the medical flat. Following this, 98.5 ml of deionised water

was added to the medical flat using a glass measuring cylinder. The medium was then mixed thoroughly. Once dissolved, the pH of the medium was adjusted to 7.6 using 0.1 M NaOH or 0.1 M HCl as appropriate. The medium was then sterilised at 116 ^{O}C for 10 minutes.

Once cooled, 3 x 20 ml amounts were aseptically poured into sterile glass universals. Amounts of 400 mg of cellobiose and trehalose were added to two of the three universals (one carbohydrate in each universal) containing 20 ml of the indicator medium. Once dissolved, the pH of the medium was adjusted to 7.6 and subsequently the medium was filter sterilised. The final universal contained the indicator medium only and was used as a substrate-free control.

In microtitre tray format, each carbohydrate was prepared as follows:
90 µl of the indicator medium containing the carbohydrate was added to 10 µl of the inoculum for each of the organisms tested. These tests were set up in duplicate. Three aliquots of 10 µl sterile distilled water were included in each tray as organism-free controls. An additional microtitre tray was set up in which the substrate-free control was also added to each organism suspension and to the organism-free controls. The trays were sealed and incubated at 30 ^{O}C over a 5 day period. At 24 hr intervals, the tray was read visually and spectrophotometrically.

b) screening organism collection B

The above methodology was adopted to screen all 282 Bcc isolates included within collection B. The following carbohydrates were evaluated: adonitol, D-Fructose,

salicine, cellobiose, maltose, trehalose, amygdaline, arbutine, ribose, D-raffinose and melibiose. A 600 mg amount of each carbohydrate was added to 30 ml of the pH indicator medium. Three 96-well microtitre trays were prepared per carbohydrate evaluated. A total of 94 test organisms were included per tray, plus one positive control, and one organism-free negative control. An additional set of three mictrotitre trays containing all 282 organisms was also set up per test run, this incorporated 90 µl of substrate-free medium included in place of carbohydrate. The positive control organisms used for each carbohydrate run were selected from those which gave strong positive reactions in the 50CH strips and are shown in table 3.2. The screening with cellobiose, trehalose and maltose was performed in duplicate.

Table 3.2: Positive control organisms used in the carbohydrate extended screen

Carbohydrate	Control organism
Adonitol	*B. cenocepacia* LMG 16659
D-Fructose	*B. cenocepacia* LMG 18827
Salicine	*B. cenocepacia* LMG 16659
Cellobiose	*B. stabilis* LMG 18888
Maltose	*B. cepacia* LMG 17997
Trehalose	*B. multivorans* LMG 17588
Amygdaline	*B. cepacia* LMG 2161
Arbutine	*B. cepacia* LMG 1222
Ribose	*B. cepacia* LMG 17997
D-Raffinose	*B. cepacia* LMG 17997

Evaluation of indoxyl-derivatised chromogenic substrates for potential use in the identification of Bcc strains using chromogenic strips (bioMérieux, France)

Chromogenic strips obtained from bioMérieux, France, containing various indoxylic chromogenic substrates, were used to screen 13 strains of Bcc and 13 isolates of *P.*

aeruginosa and the control strain of *E. coli*. These strips consist of 53 micro-tubes distributed over 3 separate strips, each containing a chromogenic enzyme substrate which together screen for a number of bacterial enzymes.

All strains used in this study were taken from collection A. The 13 strains of Bcc included 10 strains selected from the 30 BCCM reference panel of Bcc isolates and included at least one representative of each genomovar (LMG 1222, LMG 2161, LMG 16654, LMG 16659, LMG 13010, LMG 16660, LMG 14086, LMG 14294, LMG 10929, LMG 16232). The remaining three isolates consisted of two *B. dolosa* strains (LMG 18941, LMG 18942) and one *B. ambifaria* strain (LMG 11351).

Of the 13 *P. aeruginosa* isolates tested, 5 were microbanked (Mb) clinical isolates cultured from pre-operative BALs of CF lung transplant patients in the Microbiology Dept. of the Freeman Hospital (Mb 2688, Mb 2742, Mb 2749, Mb 2772, Mb 2775). Six strains were obtained from BSAC (PAE 1, PAE 10, PAE 20, PAE 30, PAE 40, PAE 50), and two strains were from NCTC (NCTC 6749 and NCTC 10332). *E. coli* was inoculated into one set of strips as a control organism, water was inoculated into a further set as a negative control. All strips were inoculated according to manufacturers instructions. Bacterial suspensions were prepared in sterile distilled water with turbidity equivalent to 1 McFarland (3×10^8 cfu/ml) using a densitometer. The strips were incubated at 30 ^{O}C for 24 hrs in aerobic conditions. The individual wells were recorded as positive or negative by colour changes observed, according to manufacturers instructions.

Results

Evaluation of fluorogenic and chromogenic substrates for differentiation of Bcc and closely related species – Initial screen (collection A)

Appendices 3.2-3.4 contain the initial readings taken during the screening of the 183 organisms of collection A with all fluorogenic and chromogenic substrates. Appendices 3.2 and 3.3 contain 18 hr fluorometer readings and 48 hr spectrophotometer readings respectively. Appendix 3.4 contains colour recordings observed with the chromogenic substrates after 48 hrs.

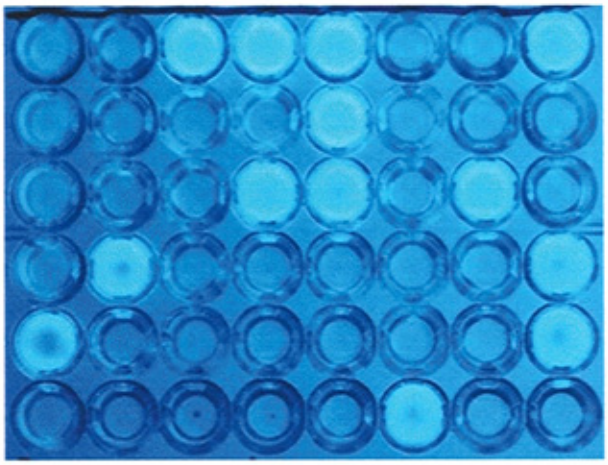
Tables 3.3 to 3.9 show the cumulative results for those substrates screened against all 183 organisms that showed the highest level of differentiation between Bcc strains and closely related species, and also between the individual species of the Bcc. The numbers in brackets represent the percentage found when the screens were performed in duplicate. For those substrates that showed less potential for discrimination, the results are recorded in Appendix 3.5. In cases where substrates gave either 100 % positive or 100 % negative reactions for all strains tested, data are not recorded in table format.

Fluorogenic substrates

Figure 3.8 illustrates an example of the reactions occurring in the microtitre wells containing fluorogenic 4-methylumbelliferyl-linked substrates, showing results in the presence and absence of the hydrolysing enzyme. The fluorescing wells (enzyme

present) are emitting a fluorescent increase reading of > 1000, the non-fluorescing wells (no enzyme present) all have a reading of < 200.

Figure 3.8: Section of microtitre tray illustrating wells containing fluorogenic substrate (core molecule linked to MU), showing both positive (fluorescent) and negative (non-fluorescent) reactions



Glycoside substrates

Table 3.3 shows the cumulative results for the glycoside substrates tested which gave the greatest discrimination. Of all the glycoside substrates evaluated, hydrolysis of MU-α-L-arabinopyranoside, MU-ß-D-galactopyranoside, MU-α-D-glucopyranoside, MU-ß-D-glucuronide, MU-α-D-mannopyranoside and MU-ß-D-mannopyranoside were shown to be of little discriminative use after the first screen and so were not tested any further (see Appendix 3.5). The remaining glycosides were potentially discriminatory, these substrates were therefore tested in duplicate to ensure reproducibility could be achieved. These duplicate tests showed that hydrolysis of MU-ß-cellobioside and MU-α-glucuronide was not reproducible and so these substrates were not tested any further.

Table 3.3: Cumulative percentages of organisms hydrolysing glyosidase enzyme substrates

Organism	No. of strains	Glycosidase substrate							
		MU-ß-cel	MU-ß-fuc	MU-α-gal	MU-ß-glu	MU-ß-nag	MU-α-gur	MU-ß-rib	MU-ß-xyl
Acinetobacter sp.	7	0 (0)	0 (0)	0 (0)	0 (0)	14 (0)	43 (0)	0 (14)	0 (14)
Brevindumonas sp.	2	50 (50)	0 (0)	0 (0)	50 (50)	50 (0)	0 (0)	100 (100)	0 (0)
Bcc	33	6 (36)	42 (39)	21 (18)	58 (64)	70 (67)	12 (0)	82 (61)	82 (100)
Bcc species									
B. cepacia	4	25 (50)	75 (75)	75 (50)	100 (100)	100 (100)	0 (0)	100 (50)	100 (100)
B. multivorans	8	0 (38)	50 (38)	0 (0)	25 (38)	38 (38)	13 (0)	100 (38)	88(100)
B. cenocepacia	10	0 (50)	70 (50)	20 (20)	100 (90)	90 (90)	0 (0)	100 (80)	100 (100)
B. stabilis	4	25 (25)	25 (25)	50 (75)	50 (75)	75 (100)	25 (0)	75 (100)	100 (100)
B. vietnamiensis	4	0 (0)	0 (0)	0 (0)	0 (0)	25 (50)	50 (0)	0 (0)	0(100)
B. dolosa	2	0 (50)	0 (50)	0 (50)	0 (100)	100 (50)	0 (0)	50 (100)	50 (50)
B. ambifaria	1	0 (0)	0 (0)	0 (0)	100 (100)	100 (100)	0 (0)	100 (100)	100 (100)
Burkholderia gladioli	6	0 (0)	0 (0)	0 (0)	0 (0)	100 (100)	0 (0)	100 (100)	33 (50)
other *Burkholderia* sp.	9	0 (25)	33 (13)	33 (25)	11 (38)	11 (25)	0 (0)	56 (88)	22 (38)
C. meningosepticum	1	100 (0)	0 (0)	100 (100)	100 (100)	100 (100)	0 (0)	0 (0)	1 (0)
Moraxella sp	3	0 (0)	0 (0)	33 (0)	67 (0)	67 (0)	0 (0)	67 (100)	2 (0)
Oligella sp.	1	0 (0)	0 (0)	0 (0)	100 (0)	100 (0)	0 (0)	0 (100)	3 (0)
Pandoraea sp.	8	0 (13)	0 (0)	0 (38)	0 (38)	0 (38)	0 (0)	25 (25)	0 (0)
P. aeruginosa	74	1 (1)	0 (1)	0 (0)	4 (14)	3 (1)	1 (0)	100 (100)	1 (0)
other *Pseudomonas* sp.	17	18 (18)	12 (6)	18 (18)	29 (29)	29 (24)	0 (0)	83 (76)	6 (6)
Ralstonia pickettii	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
other *Ralstonia* sp.	16	6 (6)	0 (0)	0 (19)	6 (19)	0 (19)	0 (0)	25 (13)	0 (6)
S. spiritivorum	1	100 (100)	0 (0)	100 (100)	100 (100)	100 (100)	100 (0)	0 (100)	100 (100)
S. maltophilia	2	100 (50)	0 (0)	50 (100)	100 (100)	100 (100)	0 (0)	100 (100)	0 (100)

(#): percentages from duplicate runs

Key:

MU-ß-cel, 4-methylumbelliferyl-ß-D-cellobioside; MU-ß-fuc, 4-methylumbelliferyl-ß-D-fucoside; MU-α-gal, 4-methylumbelliferyl-α-D-galactopyranoside; MU-ß-glu, 4-methylumbelliferyl-ß-D-glucopyranoside; MU-ß-nag, 4-methylumbelliferyl-N-acetyl-ß-D-glucosaminide; MU-α-gur, 4-methylumbelliferyl-α-D-glucuronide; MU-ß-rib, 4-methylumbelliferyl-ß-D-ribofuranoside; MU-ß-xyl, 4-methylumbelliferyl-ß-D-xylopyranoside.

Some of the substrates could differentiate a Bcc strain from other closely related organisms. Production of ß-ribosidase or ß-xylosidase, i.e. hydrolysis of the substrate MU-ß-riboside or MU-ß-xyloside could potentially identify an organism as being a Bcc member, as opposed to *Pandoraea* sp. or *R pickettii*, of which few strains produced these enzymes. Production of ß-ribosidase could also differentiate a *Pandoraea* sp. or *R. pickettii* strain from *B. gladioli*. ß-xylosidase was found to be produced by 82-100 % of Bcc strains but only 0-1 % *P. aeruginosa* strains.

Some of the species within the Bcc complex showed variation in their production of glycosidases. Although the enzyme ß-cellobiosidase was produced by only a low percentage of Bcc strains, *B. vietnamiensis* and *B. ambifaria* were the only species that were uniformly negative for production of this enzyme, although the availability of only one isolate of *B. ambifaria* means that definitive conclusions cannot be drawn. Hydrolysis of MU-ß-fucoside, i.e. production of the enzyme ß-fucosidase, was similarly observed in a proportion of all Bcc species except *B. vietnamiensis* and *B. ambifaria*. Production of the enzyme α-galactosidase, (hydrolysis of MU-α-galactopyranoside) was observed in a proportion of *B. cepacia*, *B. cenocepacia*, *B. stabilis* and *B. dolosa* isolates, but not in any strains of *B. multivorans*, *B. vietnamiensis* or *B. ambifaria*. Production of ß-glucosidase was not observed in any *B. vietnamiensis* strains, but found in all *B. cepacia* strains, the majority of *B. cenocepacia* strains, and in the single *B. ambifaria* strain tested. The enzyme N-acetyl-ß-D-glucosaminidase was produced by all strains of *B. cepacia* and the single strain of *B. ambifaria*. ß-ribosidase was produced by a proportion of all Bcc species tested except by *B. vietnamiensis* strains.

Aminopeptidase substrates

Table 3.4 shows the cumulative results for the aminopeptidase substrates tested which gave the greatest discrimination between strains. Of all the aminopeptidase substrates tested, hydrolysis of L-alanyl-AMC, L-arginyl-AMC, benzyloxycarbonyl-arginyl-AMC, L-aspartyl-AMC, asparginyl-AMC, L-glutamyl-AMC, L-glutamic acid-AMC, glycyl-AMC, L-leucyl-AMC, L-lysyl-AMC, L-phenylalanyl-AMC, L-prolyl-AMC, L-isoleucyl-AMC and L-tyrosyl-AMC showed to be of little discriminative use and so were not tested further (see Appendix 3.5). The remaining aminopeptidase substrates were potentially discriminatory, these substrates were therefore tested in duplicate to ensure reproducibility. From the results of the duplicate screen it was found that hydrolysis of ß-alanyl-AMC, glycyl-L-prolyl-AMC, L-histidyl-AMC and L-threonyl-AMC had poor reproducibility and therefore these substrates were not tested any further. Of the remaining, more useful substrates, L-pyroglutamyl-AMC hydrolysis was found to discriminate between *P. aeruginosa* isolates (positive) and Bcc strains (negative). L-valine aminopeptidase was found to be produced by all *B. gladioli* strains but by no Bcc strains. L-valyl-AMC hydrolysis also showed potential for differentiating between Bcc strains and other *Burkholderia* sp. L-ornithine aminopeptidase was produced by a proportion of all Bcc strains except *B. multivorans* and the single *B. ambifaria* strain. *B. dolosa* was the only Bcc strain to produce L-pyroglutamyl aminopeptidase.

Table 3.4: Cumulative percentages of organisms hydrolysing aminopeptidase enzyme substrates

Organism	No. of strains	Aminopeptidase substrate						
		ß-ala-AMC	gly-L-pro-AMC	L-his-AMC	L-orn-AMC	L-pyr-AMC	L-thr-AMC	L-val-AMC
Acinetobacter sp.	7	43 (0)	0 (0)	29 (57)	29 (43)	43 (14)	29 (57)	71 (71)
Brevindumonas sp.	2	100 (0)	100 (100)	100 (100)	50 (100)	0 (0)	100 (100)	100 (100)
Bcc	33	58 (9)	42 (55)	67 (21)	15 (6)	6 (3)	27 (42)	6 (3)
Bcc species								
B. cepacia	4	100 (0)	75 (75)	50 (25)	25 (25)	0 (0)	25 (0)	25 (0)
B. multivorans	8	13 (0)	0 (38)	88 (0)	0 (0)	0 (0)	50 (63)	0 (0)
B. cenocepacia	10	70 (0)	60 (90)	70 (20)	10 (0)	0 (0)	20 (60)	0 (0)
B. stabilis	4	50 (50)	25 (0)	25 (0)	25 (0)	0 (0)	25 (0)	0 (0)
B. vietnamiensis	4	50 (25)	25 (0)	25 (50)	50 (0)	0 (0)	25 (25)	0 (0)
B. dolosa	2	50 (50)	100 (100)	50 (50)	50 (50)	50 (50)	0 (100)	50 (0)
B. ambifaria	1	100 (100)	100 (100)	100 (100)	0 (0)	(0) (0)	0 (0)	0 (100)
Burkholderia gladioli	6	0 (0)	50 (33)	83 (83)	50 (67)	50 (50)	83 (83)	100 (100)
other *Burkholderia* sp.	9	44 (25)	44 (50)	67 (88)	33 (63)	38 (25)	56 (100)	67 (100)
C. meningosepticum	1	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Moraxella sp	3	67 (100)	67 (100)	100 (33)	100 (100)	100 (67)	100 (100)	33 (0)
Oligella sp.	1	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Pandoraea sp.	8	0 (25)	0 (50)	0 (63)	0 (50)	0 (25)	0 (50)	50 (63)
P. aeruginosa	74	23 (3)	1 (70)	93 (18)	61 (64)	96 (96)	51 (86)	1 (0)
other *Pseudomonas* sp.	17	71 (47)	41 (41)	94 (76)	65 (71)	35 (47)	82 (59)	47 (65)
Ralstonia pickettii	4	25 (25)	25 (25)	75 (100)	50 (0)	75 (25)	100 (100)	75 (25)
other *Ralstonia* sp.	16	13 (19)	0 (50)	50 (56)	19 (38)	63 (81)	56 (94)	44 (38)
S. spiritivorum	1	100 (0)	100 (100)	100 (100)	100 (100)	0 (0)	100 (100)	100 (100)
S. maltophilia	2	0 (0)	100 (100)	100 (100)	0 (0)	0 (0)	100 (100)	100 (100)

(#): percentages from duplicate runs

Key:

ß-ala-AMC, ß-alanyl-7-amido-4-methylcoumarin; gly-L-pro-AMC, glycine-L-prolyl-7-amido-4-methylcoumarin;L-his-AMC, L-histidyl-7-amido-4-methylcoumarin; L-orn-AMC, L-ornithinyl-7-amido-4-methylcoumarin; L-pyr-AMC, L-pyroglutamyl-7-amido-4-methylcoumarin; L-thr-AMC, L-threonyl-7-amido-4-methylcoumarin; L-val-AMC, L-valine- 7-amido-4-methylcoumarin.

Esterase substrates

Table 3.5 shows the cumulative results for the esterase substrates tested which gave the greatest discrimination. Of all the esterase substrates tested, hydrolysis of MU-lignocerate (data not shown), MU-acetate, MU-propionate, MU-butyrate, MU-heptanoate, MU-nonanoate, MU-laurate, MU-elaidate and MU-guanidinobenzoate showed to be of little discriminative use, and so were not tested any further (see Appendix 3.5). The remaining esterase substrates were potentially discriminatory and were tested in duplicate to ensure reproducibility. From the first run it became apparent that hydrolysis of MU-palmitate, MU-oleate and MU-stearate had potential for identification of *Pandoraea* spp. as all Bcc species and the majority of all other closely related species produced the esterases hydrolysing these substrates, but *Pandoraea* strains did not produce these enzymes. From the results of the duplicate test run, it was found that the hydrolysis of MU-oleate was not reproducilble. Hydrolysis of MU-palmitate and MU-stearate was observed with all strains of *R. pickettii,* but only with a small number of other *Ralstonia* sp. Stearate esterase was produced by *P. aeruginosa* but in only a small percentage of other *Pseudomonas* spp.

Table 3.5: Cumulative percentages of organisms hydrolysing esterase enzyme substrates

Organism	No. of strains	Esterase substrate		
		MU-pal	MU-ole	MU-ste
Acinetobacter sp.	7	14 (14)	86 (86)	71 (14)
Brevindumonas sp.	2	0(0)	100 (100)	0 (0)
Bcc	33	100 (100)	100 (100)	100 (100)
Bcc species				
B. cepacia	4	100 (100)	100 (100)	100 (100)
B. multivorans	8	100 (100)	100 (100)	100 (100)
B. cenocepacia	10	100 (100)	100 (100)	100 (80)
B. stabilis	4	100 (100)	100 (100)	100 (100)
B. vietnamiensis	4	100 (100)	100 (100)	100 (100)
B. dolosa	2	100 (50)	100 (100)	100 (100)
B. ambifaria	1	100 (100)	100 (100)	100 (100)
Burkholderia gladioli	6	100 (100)	100 (100)	83 (100)
other *Burkholderia* sp.	9	67 (88)	67 (100)	67 (75)
C. meningosepticum	1	0 (0)	100 (100)	0 (0)
Moraxella sp	3	33 (100)	33 (67)	67 (67)
Oligella sp.	1	0 (100)	100 (100)	100 (0)
Pandoraea sp.	8	0 (25)	0 (63)	0 (25)
P. aeruginosa	74	97 (95)	100 (100)	99 (96)
other *Pseudomonas* sp.	17	53 (24)	88 (88)	6 (12)
Ralstonia pickettii	4	100 (100)	100 (100)	100 (100)
other *Ralstonia* sp.	16	13 (31)	56 (81)	19 (31)
S. spiritivorum	1	0 (0)	100 (100)	0 (0)
S. maltophilia	2	50 (50)	100 (100)	50 (0)

(#): percentages from duplicate runs

Key:

MU-pal, 4-methylumbelliferyl-palmitate; MU-ole, 4-methylumbelliferyl-oleate; MU-ste, 4-methylumbelliferyl-stearate.

Miscellaneous fluorogenic substrates

Of the two miscellaneous fluorogenic substrates tested, MU-sulphate hydrolysis was not observed in any strains of Bcc or closely related species. Table 3.6 shows the cumulative results for the hydrolysis of MU-phosphate. The results show that phosphatase was not produced by *R. pickettii, Acinetobacter* sp. and *P. aeruginosa* strains, but the enzyme was produced by all Bcc strains, and most closely related organisms.

Chromogenic substrates

Figure 3.9 illustrates an example of the reactions occurring in the microtitre wells containing *p*NP and *p*NA-linked substrates, showing results in the presence and absence of the hydrolysing enzyme after 48 hrs. The yellow wells (enzyme present) are producing a yellow colour which was scored ++ or +++, the colourless wells (no enzyme present) were scored -, +/-, or +.

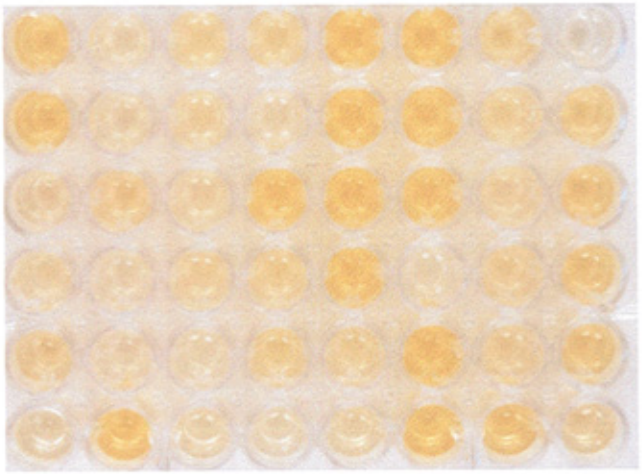
Table 3.6: Cumulative percentages of organisms hydrolysing MU-phosphate

Organism	No. of strains	MU-pho
Acinetobacter sp.	7	0 (0)
Brevindumonas sp.	2	100 (100)
Bcc	33	100 (100)
Bcc species		
B. cepacia	4	100 (100)
B. multivorans	8	100 (100)
B. cenocepacia	10	100 (100)
B. stabilis	4	100 (100)
B. vietnamiensis	4	100 (100)
B. dolosa	2	100 (100)
B. ambifaria	1	100 (100)
Burkholderia gladioli	6	100 (100)
other *Burkholderia* sp.	9	78 (100)
C. meningosepticum	1	100 (100)
Moraxella sp	3	0 (33)
Oligella sp.	1	0 (100)
Pandoraea sp.	8	25 (38)
P. aeruginosa	74	5 (9)
other *Pseudomonas* sp.	17	41 (53)
Ralstonia pickettii	4	0 (0)
other *Ralstonia* sp.	16	38 (69)
S. spiritivorum	1	100 (100)
S. maltophilia	2	100 (100)

(#): percentages from duplicate runs

Key:
MU-pho, 4-methylumbelliferyl-phosphate

Figure 3.9: section of microtitre tray illustrating wells containing chromogenic substrate (core molecule linked to *para*-nitrophenol) showing both positive (yellow) and negative (colourless) reactions



Endopeptidase substrates

Table 3.7 shows the cumulative results for all endopeptidase substrates tested which showed potential discrimination. Of all the substrates tested, hydrolysis of Ac-ala-ala-ala-*p*NA, Ac-phe-*p*NA, Ac-met-AMC (data not shown), Suc-ala-ala-pro-phe-*p*NA, H-gly-phe-*p*NA, Boc-leu-gly-arg-AMC, Ac-ala-ala-pro-ala-*p*NA and Bz-DL-arg-*p*NA (see Appendix 3.5) were shown to be of little discriminative value, and so were not tested any further (see Materials section for full substrate names).

Table 3.7: Cumulative percentages of organisms hydrolysing endopeptidase enzyme substrates

Organism	No of strains	Endopeptidase substrate			
		H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	H-ala-ala-phe-*p* NA	H-cys(bzl)-*p* NA
Acinetobacter sp.	7	14 (14)	0 (0)	43 (0)	0 (0)
Brevindumonas sp.	2	100 (100)	100 (100)	100 (100)	0 (0)
Bcc	33	21 (21)	24 (3)	18 (3)	12 (0)
Bcc strains					
B. cepacia	4	0 (0)	50 (0)	0 (0)	0 (0)
B. multivorans	8	38 (38)	38 (0)	38 (0)	13 (0)
B. cenocepacia	10	30 (30)	20 (0)	10 (10)	0 (0)
B. stabilis	4	0 (25)	0 (0)	25 (0)	0 (0)
B. vietnamiensis	4	0 (0)	0 (0)	0 (0)	50 (0)
B. dosola	2	50 (0)	0 (0)	50 (0)	50 (0)
B. ambifaria	1	0 (0)	100 (100)	0 (0)	0 (0)
Burkholderia gladioli	6	17 (0)	0 (0)	0 (50)	17 (0)
other *Burkholderia* sp.	8	50 (63)	0 (13)	50 (75)	50 (0)
C. meningosepticum	1	100 (100)	100 (100)	100 (100)	100 (100)
Moraxella sp	3	33 (0)	0 (0)	67 (67)	33 (67)
Oligella sp.	1	100 (100)	0 (0)	100 (100)	100 (100)
Pandoraea sp.	8	13 (0)	0 (0)	13 (38)	13 (25)
P. aeruginosa	74	62 (74)	12 (8)	28 (41)	0 (0)
other *Pseudomonas* sp.	17	71 (59)	24 (18)	59 (47)	12 (12)
Ralstonia picketti	4	25 (0)	0 (0)	0 (0)	0 (0)
other *Ralstonia* sp.	16	38 (19)	6 (0)	38 (25)	31 (13)
S. spiritivorum	1	100 (100)	0 (0)	100 (0)	0 (0)
S. maltophilia	2	100 (100)	0 (100)	0 (0)	0 (0)

(#): percentages from duplicate runs

Table 3.7 (cont'd..): Cumulative percentages of organisms hydrolysing endopeptidase enzyme substrates

Organism	No of strains	Endopeptidase substrate		
		Suc-ala-ala-ala-*p* NA	Suc-ala-leu-pro-phe-*p* NA	H-y-glu-ala-gly-*p* Na
Acinetobacter sp.	7	0 (0)	0 (0)	0 (0)
Brevindumonas sp.	2	100 (50)	100 (100)	0 (0)
Bcc	33	9 (0)	12 (12)	9 (3)
Bcc strains				
B. cepacia	4	0 (0)	0 (25)	0 (0)
B. multivorans	8	25 (0)	25 (13)	0 (0)
B. cenocepacia	10	10 (0)	20 (20)	0 (10)
B. stabilis	4	0 (0)	0	0 (0)
B. vietnamiensis	4	0 (0)	0	25 (0)
B. dolosa	2	0 (0)	0	100 (0)
B. ambifaria	1	0 (0)	0	0 (0)
Burkholderia gladioli	6	0 (0)	33 (0)	0 (0)
other *Burkholderia* sp.	8	13 (13)	63 (13)	50 (25)
C. meningosepticum	1	100 (100)	100 (0)	100 (100)
Moraxella sp	3	0 (0)	67 (0)	33 (33)
Oligella sp.	1	100 (0)	100 (0)	100 (100)
Pandoraea sp.	8	0 (0)	0 (0)	0 (50)
P. aeruginosa	74	36 (9)	51 (24)	8 (12)
other *Pseudomonas* sp.	17	18 (29)	53 (29)	24 (18)
Ralstonia pickettii	4	0 (25)	75 (75)	0 (0)
other *Ralstonia* sp.	16	0 (13)	13 (44)	19 (25)
S. spiritivorum	1	0 (0)	0 (0)	0 (0)
S. maltophilia	2	100 (100)	100 (100)	100 (50)

(#): percentages from duplicate runs

The remaining endopeptidase substrates were potentially discriminatory, these substrates were therefore tested in duplicate to ensure reproducibility. From the results of the duplicate run, the majority of the remaining substrates were found to be unreproducible. However, hydrolysis of H-glu-gly-arg-*p*NA and suc-phe-leu-phe-*p*NA was found to be sufficiently reproducible. The substrate H-glu-gly-arg-pNA (a substrate for the endopeptidase urokinase) was hydrolysed by a percentage of all Bcc species except *B. cepacia, B. vietnamiensis* and the single *B. ambifaria* strain. Suc-phe-leu-phe-*p*NA (a substrate for the endopeptidase cathepsin G) was hydrolysed by all Bcc species except *B. stabilis*, *B. vietnamiensis*, or *B. dolosa*

Miscellaneous chromogenic substrates

Table 3.8 shows the cumulative results for all miscellaneous chromogenic substrates tested which showed potential discrimination. Of all the substrates tested, hydrolysis of *p*NP-ß-L-arabinopyranoside, *p*NP-α-L-rhamnopyranoside, *p*NP-α-D-xylopyranoside (data not shown), *p*NP caprylate, *p*NP-α-L-fucoside, *p*NP-ß-D-lactopyranoside and *p*NP-valerate showed to be of little discriminative use and so were not tested any further (see Appendix 3.5). The remaining nitrophenyl linked substrates were potentially discriminatory, these substrates were therefore tested in duplicate to ensure reproducibility. From the results of the duplicate run, it was found that hydrolysis of *p*NP-caprate, *p*NP-phosphorylcholine, *p*NP-α-D-maltoside and *p*NP-ß-maltoside was not reproducible.

Table 3.8: Cumulative percentages of organisms hydrolysing miscellaneous chromogenic enzyme substrates.

Organism	No of strains	miscellaneous substrates			
		*p*NP-caprate	*p*NP-p`-guanidinobenzoate	*p*NP-myristate	*p*NP-phosphorylcholine
Acinetobacter sp.	7	100 (100)	0	43 (14)	43 (14)
Brevindumonas sp.	2	100 (100)	0	50	0 (0)
Bcc	33	94 (100)	0	97 (97)	67 (48)
Bcc strains					
B. cepacia	4	100 (100)	0	100 (100)	100 (75)
B. multivorans	8	100 (100)	0	100 (100)	38 (38)
B. cenocepacia	10	100 (100)	0	100 (100)	100 (60)
B. stabilis	4	100 (100)	0	100 (100)	75 (100)
B. vietnamiensis	4	100 (100)	0	100 (100)	25 (0)
B. dosola	2	0 (100)	0	50 (50)	0 (0)
B. ambifaria	1	100 (100)	0	100 (100)	100 (0)
Burkholderia gladioli	6	83 (100)	0	100 (100)	100 (100)
other *Burkholderia* sp.	8	88 (100)	0	50 (50)	25 (25)
C. meningosepticum	1	100 (100)	0	0 (0)	0 (0)
Moraxella sp	3	100 (100)	0	67 (33)	67 (0)
Oligella sp.	1	100 (100)	0	100 (100)	100 (0)
Pandoraea sp.	8	75 (50)	0	0 (13)	0 (0)
P. aeruginosa	74	100 (100)	0	100 (97)	84 (73)
other *Pseudomonas* sp.	17	94 (94)	0	41 (53)	35 (12)
Ralstonia picketti	4	100 (100)	75	100 (75)	0 (0)
other *Ralstonia* sp.	16	100 (100)	31	63 (25)	13 (0)
S. spiritivorum	1	100 (100)	0	0 (100)	0 (0)
S. maltophilia	2	100 (100)	0	50 (0)	0 (0)

(#): percentages from duplicate runs

Table 3.8 (cont`d..): Cumulative percentages of organisms hydrolysing miscellaneous chromogenic enzyme substrates

Organism	No of strains	miscellaneous substrates		
		p NP-phenyl-phospho	*p* NP-α-D-malto	*p* NP-ß-D-malto
Acinetobacter sp.	7	0 (0)	0 (0)	0 (0)
Brevindumonas sp.	2	0 (0)	50 (50)	0 (0)
Bcc	33	6 (6)	6 (0)	6 (0)
Bcc strains				
B. cepacia	4	0 (0)	0 (0)	0 (0)
B. multivorans	8	0 (0)	0 (0)	0 (0)
B. cenocepacia	10	0 (0)	0 (0)	0 (0)
B. stabilis	4	0 (0)	0 (0)	0 (0)
B. vietnamiensis	4	0 (0)	0 (0)	0 (0)
B. dosola	2	100 (100)	100 (0)	100 (0)
B. ambifaria	1	0 (0)	0 (0)	0 (0)
Burkholderia gladioli	6	0 (0)	17 (0)	0 (0)
other *Burkholderia* sp.	8	13 (25)	38 (13)	13 (0)
C. meningosepticum	1	100 (100)	100 (100)	100 (0)
Moraxella sp	3	0 (0)	0 (0)	0 (0)
Oligella sp.	1	0 (0)	100 (100)	0 (0)
Pandoraea sp.	8	13 (25)	13 (25)	0 (13)
P. aeruginosa	74	0 (0)	0 (0)	0 (0)
other *Pseudomonas* sp.	17	0 (24)	47 (29)	0 (6)
Ralstonia picketti	4	0 (0)	50 (25)	0 (0)
other *Ralstonia* sp.	16	6 (25)	25 (25)	6 (0)
S. spiritivorum	1	0 (100)	100 (100)	100 (100)
S. maltophilia	2	50 (0)	100 (100)	50 (0)

(#): percentages from duplicate runs

Key:

p NP- phenyl-phospho, *p* -nitrophenyl phenyl phosphonate; *p* NP-α-D-malto, *p* -nitrophenyl-α-D-maltopyranoside; *p* NP-ß-D-malto, *p* -nitrophenyl-ß-D-maltopyranoside

Of the remaining, more useful substrates, *p*-NP-*p*`-guanidinobenzoate was found to be hydrolysed only by *R. pickettii* strains with 75 % of isolates producing the enzyme, and 31 % of other *Ralstonia* sp., no other species tested produced the hydrolysing enzyme. Myristate esterase was not produced by *Pandoraea* sp. compared to the majority of all other species tested. All Bcc strains produced this enzyme except for one strain of *B. dolosa*. Hydrolysis of *p*NP-phenyl-phosphonate (i.e. production of 5′-nucleotide phosphodiesterase) was observed in both strains of *B. dolosa* but the substrate was not hydrolysed by any other Bcc species.

Carboxypeptidase substrates

The results from the pilot study revealed that there was little difference in the results observed for the screening for carboxypeptidase production using either the centrifuge or the non-centrifugation method. However, the purple colouration upon ninhydrin addition where the substrate had been hydrolysed was slightly more intense when using the non-centrifugation technique. Therefore, this technique was adopted for screening of the organism collection for carboxypeptidase activity. Figure 3.10 illustrates the purple coloured wells observed using the non-centrifuging method, upon the addition of ninhydrin, to the test wells. Both positive and negative reactions are present. The stronger colour illustrates +++, whilst the palest coloured wells demonstrate negative reactions. Appendix 3.6 contains the 18 hr colour recordings obtained for all carboxypeptidase substrates tested, including the duplicate test readings. All water controls were negative and therefore omitted from the tables.

Figure 3.10: Section of microtitre tray illustrating wells containing benzoyl-linked carboxypeptidase substrate after addition of ninhydrin, showing both positive (purple) and negative (colourless) reactions

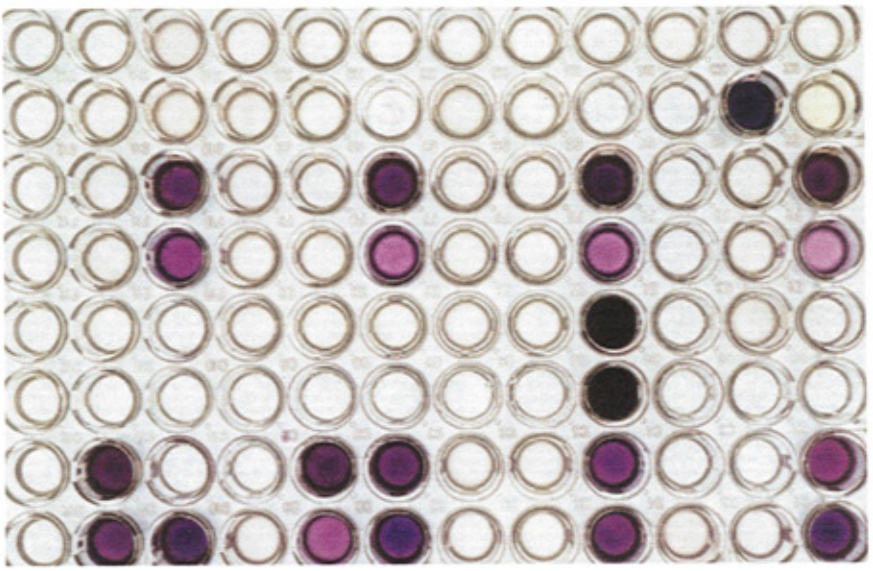


Table 3.9 shows the cumulative results for hydrolysis of all carboxypeptidase substrates evaluated. Hydrolysis of all these carboypeptidase substrates showed to be of potential discriminative use so all were performed in duplicate.

Hydrolysis of all substrates was shown to be sufficiently reproducible. Hydrolysis of benzoyl-L-phenylalanine was shown to be of little discriminative use. Alanine carboxypeptidase was produced by the majority of Bcc strains, *Pandoraea* sp., and *R. pickettii*, but only by 4 % of *P. aeruginosa* strains. *Ralstonia* sp, including *R pickettii* were among the few strains to produce glutamic carboxypeptidase. Glycine carboxypeptidase was produced by most Bcc strains and the majority of closely related organisms, but was produced by few strains *of P. aeruginosa* (8-18 %).

Table 3.9: Cumulative percentages of organisms hydrolysing carboxypeptidase enzyme substrates

Organism	No. of strains	Carboxypeptidase substrate						
		ben-L-ala	ben-L-glu	ben-gly	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
Acinetobacter sp.	7	57 (100)	43 (57)	100 (100)	71 (100)	14 (29)	71 (86)	71 (71)
Brevindumonas sp.	2	50 (100)	100 (100)	50 (100)	50 (100)	100 (100)	100 (100)	100 (100)
Bcc	33	83 (83)	3 (3)	86 (91)	51 (74)	5 (6)	80 (77)	34 (46)
Bcc species								
B. cepacia	4	75 (100)	0 (0)	75 (100)	75 (75)	0 (0)	75 (100)	75 (100)
B. multivorans	8	100 (100)	12 (13)	100 (100)	50 (88)	13 (0)	88 (100)	25 (13)
B. cenocepacia	10	70 (80)	0 (0)	90 (90)	60 (60)	0 (0)	90 (80)	40 (40)
B. stabilis	4	75 (75)	0 (0)	75 (75)	25 (75)	25 (50)	75 (75)	25 (75)
B. vietnamiensis	4	100 (50)	0 (0)	100 (100)	50 (100)	0 (0)	100 (25)	0 (25)
B. dosola	2	100 (50)	0 (0)	0 (50)	0 (0)	0 (0)	0 (50)	0 (0)
B. ambifaria	1	100 (100)	0 (0)	100 (100)	100 (100)	0 (0)	100 (100)	100 (100)
Burkholderia gladioli	6	50 (50)	0 (0)	100 (100)	0 (17)	0 (0)	83 (50)	17 (0)
other *Burkholderia* sp.	9	38 (50)	0 (0)	63 (75)	38(38)	0 (0)	50 (38)	50 (25)
C. meningosepticum	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Moraxella sp	3	33 (33)	0 (0)	33 (33)	33 (33)	0 (0)	33 (67)	33 (33)
Oligella sp.	1	0 (100)	0 (100)	0 (100)	0 (100)	0 (0)	0 (100)	0 (100)
Pandoraea sp.	8	100 (88)	13 (0)	100 (100)	0 (0)	100 (100)	100 (100)	100 (100)
P. aeruginosa	74	4 (4)	3 (3)	18 (8)	0 (1)	1 (0)	4 (0)	1 (1)
other *Pseudomonas* sp.	17	47 (53)	35 (53)	59 (47)	6 (18)	24 (24)	41 (47)	18 (29)
Ralstonia picketti	4	100 (100)	100 (100)	100 (100)	100 (75)	100 (100)	100 (100)	100 (100)
other *Ralstonia* sp.	16	94 (100)	75 (75)	94 (94)	75 (94)	88 (88)	100 (100)	94 (100)
S. spiritivorum	1	100 (100)	100 (100)	100 (100)	100 (100)	0 (0)	100 (100)	100 (100)
S. maltophilia	2	50 (100)	100 (100)	0 (0)	0 (0)	100 (100)	100 (100)	0 (0)

(#): percentages from duplicate runs

Key:

ben-L-ala, benzoyl-L-alanine; ben-L-glu, benzoyl-L-glutamic acid; ben-gly, benzoyl-glycine; ben-L-his, benzoyl-L-histidine; ben-DL-leu, benzoyl-DL-leucine;ben-DL-met, benzoyl-DL-methionine; ben-L-phe, benzoyl-L-phenylalanine.

Histidine carboxypeptidase was produced by the majority of *R. pickettii* strains (75-100 %) but by no *Pandoraea* sp. A percentage of all Bcc strains produced this enzyme except *B. dolosa*. Leucine carboxypeptidase was produced by all strains of *Pandoraea* sp. and *R. pickettii*, but was only produced by very few Bcc, *B. gladioli*, or *P. aeruginosa* strains. Methionine carboxypeptidase was found to be produced by the majority of Bcc members (77-80 %), all *Pandoraea* sp. and all *Ralstonia* sp. including *R. pickettii* strains. However, this enzyme was not produced by *P. aeruginosa*.

Evaluation of fluorogenic and chromogenic substrates for differentiation of Bcc and closely related species – Extended screen (collection B)

Appendices 3.7 and 3.8 contain the fluorogenic and chromogenic visual readings respectively for all substrates tested against all 310 strains. Table 3.10 shows the cumulative results for organisms in this collection found to hydrolyse these fluorogenic and chromogenic substrates.

Table 3.10: Cumulative percentages of organisms from Collection B hydrolysing various enzyme substrates

Organism	No of strains	MU-phos	MU-pal	MU-ß-rib	MU-ß-xyl	pyr-AMC	MU-ß-fuc	val-AMC
Acinetobacter sp	**1**	0	0	0	0	0	0	0
Alcaligenes denitrifications	**4**	0	0	0	0	0	0	0
Alcaligenes faecalis	**1**	0	0	0	0	0	0	0
Alcaligenes xylosoxidans	**7**	14	14	14	0	14	0	0
Bcc	**282**	95	90	32	31	1	2	2
Bcc species								
B. cepacia	**36**	94	97	42	69	0	6	0
B. multivorans	**48**	100	96	0	6	0	0	0
B. cenocepacia	**81**	98	81	20	27	4	1	5
B. cenocepacia III-A	**51**	98	86	20	22	6	0	8
B. cenocepacia III-B	**30**	97	73	20	37	0	3	0
B. stabilis	**32**	100	97	91	34	0	6	0
B. vietnamiensis	**42**	74	86	2	26	0	0	0
B. dolosa	**17**	100	82	76	29	0	0	0
B. ambifaria	**11**	100	100	64	73	0	0	0
B. anthina	**13**	100	100	69	0	0	0	0
B. pyroccinia	**2**	100	100	0	100	0	0	0
P. apista	**1**	0	0	0	0	0	0	0
P. pnomenusa	**2**	0	0	0	0	0	0	0
P. sputorum	**2**	0	0	0	0	0	0	0
R. pickettii	**5**	0	80	0	0	40	0	0
S. maltophilia	**5**	100	0	100	0	0	0	0

(#): percentages from duplicate runs

Key:

MU-phos, 4-methylumbelliferyl phosphate; MU-pal, 4-methylumbelliferyl palmitate; MU-ß-rib, 4-methylumbelliferyl-ß-riboside; MU-ß-xyl, 4-methylumbelliferyl-ß-xyloside; pyr-AMC, pyroglutamyl-7-amido-4-methylcoumarin; MU-ß-fuc, 4-methylumbelliferyl-ß-fucoside; val-AMC, valyl-7-amido-4-methylcoumarin

Table 3.10 (cont`d..): Cumulative percentages of organisms from Collection B hydrolysing various enzyme substrates

Organism	No of strains	MU-α-gal	MU-ß-glu	*p* NP-phen-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*pNP*- p`-guan
Acinetobacter sp	**1**	0	0	0	0	0	0
Alcaligenes denitrifications	**4**	0	0	0	0	0	0
Alcaligenes faecalis	**1**	0	0	0	0	0	0
Alcaligenes xylosoxidans	**7**	0	0	0	0	0	0
Bcc	**282**	13	32	73	4	6	0
Bcc species							
B. cepacia	**36**	31	78	83	3	6	0
B. multivorans	**48**	0	0	73	2	2	0
B. cenocepacia	**81**	4	37	70	6	7	0
B. cenocepacia III-A	**51**	6	31	71	2	8	0
B. cenocepacia III-B	**30**	0	47	70	13	7	0
B. stabilis	**32**	72	44	94	3	16	0
B. vietnamiensis	**42**	0	17	62	2	2	0
B. dolosa	**17**	0	0	44	0	0	0
B. ambifaria	**11**	0	91	82	9	9	0
B. anthina	**13**	0	0	92	0	0	0
B. pyroccinia	**2**	0	100	50	0	0	0
P. apista	**1**	0	0	0	0	0	0
P. pnomenusa	**2**	0	0	0	0	0	0
P. sputorum	**2**	0	0	0	0	0	0
R. pickettii	**5**	0	0	0	0	20	100
S. maltophilia	**5**	80	80	80	100	40	0

(#): percentages from duplicate runs

Key:

MU-α-gal, 4-methylumbelliferyl-α-D-galactoside; MU-ß-glu, 4-methylumbelliferyl-ßglucoside; *p* NP-phen-phos, *p* -nitrophenyl phenyl phosphonate; *p* NP- p`-guan, *p* -nitrophenyl p`-guanidinobenzoate

In comparison with the screening of collection A of 183 organisms, there was no confirmation of the discriminatory potential of hydrolysis of the substrates MU-ß-fucoside and L-val-AMC. Hydrolysis of MU-ß-riboside also did not correlate well in comparison with collection A, although it did confirm the lack of ß-ribosidase production in strains of *B. vietnamiensis*, *Pandoraea* spp. and *R. pickettii* compared to production of the enzyme in Bcc strains (61-82 % in screening of collection A and 32 % in screening of collection B). ß-ribosidase was produced in a proportion of all Bcc strains except *B. multivorans* and *B. pyrrocinia*, and in only one strain of *B. vietnamiensis*. The extended screen (collection B) also revealed the lack of production of ß-ribosidase in *Alcaligenes* sp. Hydrolysis of MU-ß-xyloside did not correlate very well to the results found in the screening of collection A although these tests confirmed the lack of ß-xylosidase production in strains of *Pandoraea* sp. and *R. pickettii* (both 0 %) compared to the activity seen in Bcc strains (82-100 % in screening of collection A and 31 % in collection B). Screening of collection B again revealed the lack of production of the enzyme in *Alcaligenes* sp. ß-xylosidase was produced by a percentage of all Bcc species except *B. anthina.*

No strains of *B. dolosa* produced the enzyme pyroglutamyl aminopeptidase. This eliminated hydrolysis of this substrate for assisting in identification of Bcc isolates as *B. dolosa*, as was initially thought possible. *B. cenocepacia* III-A was the only strain to produce the enzyme though this percentage was very low (6 % i.e. only one strain out of 51).

Hydrolysis of MU-α-galactoside observed in the extended screen was found to correlate well to that observed in the initial sceen. Both sets of results showed *B. cepacia* and *B.*

cenocepacia to produce α-galactosidase, although the enzyme production was more common in collection A (50-75 % and 20 % respectively) than in collection B (31 % and 4 %). Both screens also showed a high percentage of *B. stabilis* isolates to produce the enzyme. Both screens revealed an absence of α-galactosidase in *B. multivorans, B. vietnaiensis* or *B. ambifaria* strains. The extended screen confirmed the absence of this enzyme in *B. dolosa* strains and also showed that *B. anthina* and the two strains of *B. pyrrocinia* and did not produce the enzyme. This therefore confirms that hydrolysis of the substrate MU-α-galactopyranoside would indicate a Bcc strain to be *B. cepacia, B. cenocepacia*, or *B. stabilis*. This extended screen confirmed absence of production of this enzyme in *P. aeruginosa*, *Pandoraea* sp. and *Ralstonia* sp., and showed that *Alcaligenes* sp. also do not produce the enzyme.

Hydrolysis of MU-ß-glucopyranoside also showed good correlation between the two screens. Both sets of results showed a high percentage of *B. cepacia* and *B. ambifaria* producing the enzyme ß-glucosidase. Both screens showed *B. cenocepacia* strains produce the enzyme. The initial screen showed 90-100 % of isolates to produce the enzyme, however, in the extended screen, only 37 % of *B. cenocepacia* isolates hydrolysed the substrate. MU-ß-glucopyranoside was not hydrolysed by any *B. multivorans* strains in collection B, and although there were some *B. multivorans* strains in collection A which produced the enzyme, the percentage was quite low (25-38 %, i.e. 2-3 organisms). The substrate was not hydrolysed by any *B. vietnamiensis* strains initially, and although the enzyme was produced by some *B. vietnamiensis* strains in the extended screen, the percentage was low (17 %). The extended screen confirmed lack of ß-glucosidase activity in *B. dolosa* strains. Both screens showed that the majority of *B. ambifaria* strains produce ß-glucosidase (only one strain was found which did not

produce the enzyme). *B. anthina* strains did not produce the enzyme but both strains of *B. pyrrocinia* did. The combination of these results suggest therefore that detection of hydrolysis of MU-ß-glucopyranoside in a Bcc isolate could eliminate *B. vietnamiensis*, *B. dolosa* and *B. anthina*, as possible species, and that a lack of hydrolysis could eliminate *B. ambifaria*. Once again, the extended screen confirmed lack of enzyme activity in *Pandoraea* spp. and *R. pickettii*, and showed that *Alcaligenes* spp. also do not produce the enzyme.

The pattern of MU-palmitate hydrolysis found with the extended screen confirmed the high palmitate esterase activity found in all species of Bcc (100 % of isolates in initial screen, 90 % in extended), and the lack of this enzyme activity in *Pandoraea* sp. The extended screen also confirmed the high palmitate esterase activity observed within *R. pickettii* strains. The hydrolysis of palmitate esterase was therefore confirmed to be suitable for the identification of *Pandoraea* spp. The extended screen also showed that *Alcaligenes* sp do not produce this enzyme.

The hydrolysis of MU-phosphate confirmed the high percentage of phosphatase activity found in all genomovars of *B. cepacia* (100 % in initial screen, 95 % in extended) compared to the lack of activity found in *Pandoraea* spp. and *R. pickettii*. The extended screen also demonstrated *Alcaligenes* spp. do not produce phosphatase. This substrate could differentiate between a Bcc strain, and the majority of other closely related organisms, i.e. *P. aeruginosa*, *Acinetobacter* spp., *Pandoraea* spp., *R. pickettii* and *Alcaligenes* spp.

The hydrolysis of H-glu-gly-arg-*p*NA (by urokinase) was observed in very few strains in the extended screen so the potential Bcc species differentiation shown in the initial screen was not confirmed. However, it was found that 100 % of *S. maltophilia* strains produced this enzyme compared to few other species screened, therefore this substrate could be useful for identification of this organism. Hydrolysis of Suc-phe-leu-phe-*p*NA was also seen in only a few organisms and was therefore deemed not to be of any discriminatory use.

The only organism which hydrolysed *p*NP-*p*`-nitroguanidinobenzoate was *R. pickettii,* all such strains produced the enzyme. This confirmed the results from the screening of collection A. Production of 5′nucleotide phosphodiesterase (detected by *p*NP-phenyl-phosphonate hydrolysis) was not confirmed as being unique to *B. dolosa,* within the Bcc. A high percentage of all Bcc strains produced the enzyme. *R. pickettii, Pandoraea* sp. and *Alcaligenes* sp. did not produce 5′nucleotide phosphodiesterase.

Initial evaluation of oxidative tests for differentiation of Bcc and closely related species using bioMérieux 50CH strips

Figure 3.11 illustrates wells from the 50CH strips giving indication of carbohydrate oxidation (yellow) and indicating absence of carbohydrate oxidation (red). Appendix 3.9 contains the original scores assigned for all reactions recorded in the strips.

Figure 3.11: wells of 50CH strip illustrating positive (yellow) and negative (red) oxidation reactions

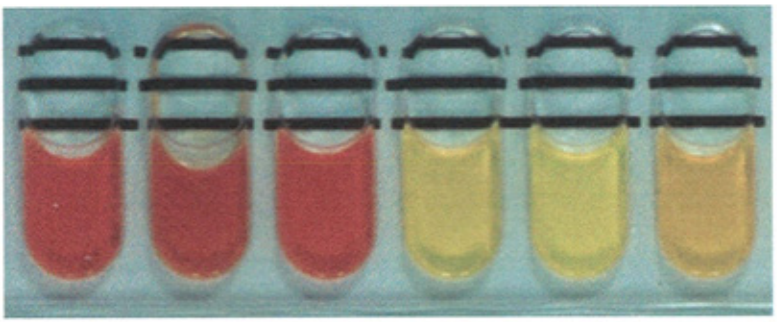


Table 3.11 shows the cumulative results for these tests which showed potential discrimination between the genomovars. The numbers represent the percentage of strains scoring 3-5 (a positive result according to manufacturer`s instructions). The numbers in brackets represent the percentage of strains scoring 1-5, i.e. when any slight colour change from red to orange was recorded as a positive reaction. This was performed to see if discrimination could be improved.

Table 3.11: Cumulative percentages of organisms oxidising carbohydrates on the 50CH strips

Bcc species	No of strains	Carbohydrate substrate								
		Glycerol	D-Arabinose	L-Arabinose	Ribose	D-Xylose	L-Xylose	Adonitol	Mannitol	D-Tagatose
B. cepacia	4	0 (25)	25 (75)	25 (100)	25 (75)	25 (75)	0	75 (100)	100	0
B. multivorans	8	38 (88)	25 (100)	88 (100)	0 (50)	88 (100)	0 (25)	63 (88)	75 (88)	0 (25)
B. cenocepacia	10	40	40 (90)	60 (100)	10 (30)	50 (100)	0 (20)	20 (70)	70 (100)	0
B. stabilis	4	25 (50)	50 (100)	50 (100)	0	50 (75)	0 (25)	50 (75)	75	0
B. vietnamiensis	4	25 (50)	25 (75)	50 (100)	0 (25)	25 (100)	0	0 (50)	50 (100)	0

(#): percentages where orange colouration recorded as indicative of oxidation

Table 3.11 (cont'd..): Cumulative percentages of organisms oxidising carbohydrates on the 50CH strips

Bcc species	No of strains	Carbohydrate substrate								
		D-Fructose	D-Mannose	Rhamnose	Dulcitol	Inositol	Arbutin	Esculin	Salicin	D-Lyxose
B. cepacia	4	25 (100)	75 (100)	25	50 (100)	100	100	50	50 (100)	0 (75)
B. multivorans	8	63 (100)	88 (100)	0	63	63 (75)	0 (13)	13	0 (13)	0 (25)
B. cenocepacia	10	40 (70)	60 (100)	0	10 (50)	80	60 (70)	70 (80)	30 (60)	0 (10)
B. stabilis	4	100	100	0	75	75 (100)	0 (50)	50	0	0
B. vietnamiensis	4	75 (100)	100	0	25 (50)	50 (100)	0	0	0	0 (25)

(#): percentages where orange colouration recorded as indicative of oxidation

Table 3.11 (cont`d..): Cumulative percentages of organisms oxidising carbohydrates on the 50CH strips

Bcc species	No of strains	Carbohydrate substrate							
		ß-Methyl-xyloside	Sorbitol	Amygdalin	Melibiose	Saccharose	Trehalose	D-Raffinose	Xylitol
B. cepacia	4	0	100	25 (75)	0 (25)	100	0 (75)	25 (50)	0
B. multivorans	8	0 (38)	63 (75)	0	0	38	63 (75)	0	0
B. cenocepacia	10	0	50 (80)	0 (10)	0 (10)	80	10 (50)	0 (10)	0
B. stabilis	4	0 (50)	75	0 (25)	25	50 (75)	100	25	25
B. vietnamiensis	4	0	50 (100)	0	0	50 (75)	50	0	0

(#): percentages where orange colouration recorded as indicative of oxidation

Table 3.11 (cont`d..): Cumulative percentages of organisms oxidising carbohydrates on the 50CH strips

Bcc species	No of strains	Carbohydrate substrate							
		Cellobiose	Maltose	Lactose	ß-Gentiobiose	D-Fucose	L-Fucose	D-Arabitol	L-Arabitol
B. cepacia	4	25 (100)	25 (100)	100	0	75 (100)	25 (50)	25 (50)	25 (75)
B. multivorans	8	38 (75)	38 (75)	50 (75)	0	100	0 (50)	38 (63)	13 (50)
B. cenocepacia	10	20 (60)	30 (70)	60 (80)	0	90 (100)	0 (50)	0 (30)	0 (20)
B. stabilis	4	100	100	100	50	75	25 (75)	25 (50)	25 (50)
B. vietnamiensis	4	0 (75)	25 (50)	75 (100)	0	50 (100)	0	25	0

(#): percentages where orange colouration recorded as indicative of oxidation

Of the carbohydrate substrates tested, oxidation of following substrates (both when scored 3-5, and 1-5) showed little or no discrimination between the five genomovars and so are not included in Table 3.11; erythritol, L-sorbose, α-methyl-D-mannoside, α-methyl-D-glucoside, N acetyl glucosamide, inulin, melezitose, amidon, glycogene, D-turanose, 5 keto-gluconate, gluconate, 2 keto-gluconate. Galactose and D-glucose were oxidised by 100 % of strains and so are not included in the results table. L-xylose, ß-methyl-xyloside, D-lyxose, and D-tagatose were oxidised by a proportion of strains but only when a result was deemed positive with a score 1-5, i.e., the reactions were weak. Little discrimination could be obtained with these figures, although a score of 1-5 with ß-methyl-xyloside would indicate a Bcc strain was either *B. multivorans* or *B. stabilis*. Oxidation of D- and L-arabinose was shown to provide little discrimination between any of the Bcc species.

There were no carbohydrates, the oxidation of which could definitively identify an isolate as a particular species of the Bcc. However, some useful results were observed, which perhaps in combination could assist Bcc species identification. Only *B. cepacia* and *B. cenocepacia* isolates oxidised ribose, arbutin, and salicin (scored 3-5). Only *B. cepacia* oxidised amygdalin and rhamnose. *B. stabilis* was the only species to oxidise melibiose, xylitol, and ß-gentiobiose (scored 3-5). *B. cepacia* and *B. stabilis* were the only strains to oxidise D-raffinose and L-fucose. There were a number of Bcc species which were the only species not to oxidise particular carbohydrates and could perhaps be identified as a result of these negative reactions. For example, *B. cepacia* did not oxidise glycerol or trehalose, *B. vietnamiensis* did not hydrolyse esculin, *B. cenocepacia* did not oxidise D-arabitol, and *B. cenocepacia* and *B. vietnamiensis* did not oxidise L-arabitol. *B. cepacia* was the only Bcc species of which 100 % of strains oxidised

mannitol, sorbitol, saccharose and inositol. *B. stabilis* was the only Bcc species of which 100 % of strains oxidised D-fructose and maltose. Similarly 100 % of *B. stabilis* and *B. vietnamiensis* strains oxidised D-mannose, 100 % of *B. cepacia* and *B. stabilis* strains oxidised lactose, and all *B. multivorans* strains oxidised D-fucose.

Extended evaluation of oxidative tests for differentiation of Bcc and closely related species reproducing bioMérieux strips - Initial investigation

Table 3.12 shows the percentages of strains oxidising the carbohydrates after 48 hours incubation.

Table 3.12: Percentages of strains oxidising carbohydrates in pilot study

Bcc species	No. of strains	Cellobiose	Trehalose
B. cepacia	3	0 (0)	0 (0)
B. multivorans	4	75 (75)	75 (75)
B. cenocepacia	5	40 (40)	40 (40)
B. stabilis	1	100 (100)	100 (100)
B. vietnamiensis	1	0 (0)	0 (0)

(#): Percentages from duplicate runs

The results were found to be reproducible compared to the 50 CH strips. The results of this pilot study correlated well with those of the 50CH strips and clear differentiation was observed using visual colour development as differentiation between positive and negative oxidation reactions. The spectrophotometer was found to be unreliable for giving an indication of positive oxidation reactions.

Extended evaluation of oxidative tests for differentiation of Bcc and closely related species reproducing bioMérieux strips

The extended panel of 282 Bcc isolates were screened for oxidation of those carbohydrates showing greatest discrimination between the Bcc species in the 50CH strips. The visual colour recordings for all carbohydrates screened are in Appendix 3.10. The absorbance readings from this study confirmed that such a method of indication of carbohydrate oxidation was not to be relied upon, with little consistency between readings taken from the same reaction well, at different time periods. These data are therefore not documented. The following results are therefore based on visual recordings where colour change was observed in the microtitre wells from red to orange/yellow. Cumulative percentages were calculated first recording an orange coloured well as indication of carbohydrate oxidation, followed by recording such reactions as absence of oxidation. This was to see if discrimination could be improved.

Of all the strains screened, none oxidised adonitol, ribose, D-raffinose or melibiose. Salicine, amygdaline and arbutine were oxidised by a small percentage of isolates and these results were of no discriminatory use. A number of strains oxidised D-fructose but again the results were of little discriminatory use. Cellobiose, maltose and trehalose oxidation showed greatest potential for discrimination between Bcc strains. The extended panel of Bcc strains were therefore tested in duplicate for oxidation of these three carbohydrates to ensure reproducibility. The cumulative results for the carbohydrate oxidation reactions are in Tables 3.13-3.15 and are shown at 24 hr intervals from 48 hrs up to 5 days. The results are not shown at 24 hrs as very little colour change was observed in any of the microtitre wells at this stage.

After 48 hrs, very few strains of *B. cepacia, B. stabilis, B. vietnamiensis* and *B. pyrrocinia* showed indication that they were oxidising cellobiose, particularly if an orange colour was assumed not to be an indication of oxidation. Around 30-40 % of most other species within the Bcc oxidised the carbohydrate, however, around 90 % of *B. anthina* strains oxidised cellobiose. Over the five day incubation period, the percentages of organisms showing signs of oxidising cellobiose slowly increased for all Bcc species, except the two *B. pyrrocinia* strains which remained negative. After 72 hrs almost 100 % of *B. anthina* strains oxidised cellobiose but still very few strains of *B. stabilis* and *B. vietnamiensis* showed positive results. After five days of incubation, apart from *B. pyrrocinia*, the majority of all strains had oxidised cellobiose, including all strains of *B. dolosa, B. ambifaria* and *B. anthina* in at least one test run.

Table 3.13: Cumulative percentages of strains oxidising cellobiose

Bcc species	no.	48hr		72hr		96hr		5day	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
B. cepacia	36	0 (0)	0 (0)	31 (44)	14 (22)	56 (61)	42 (53)	92 (75)	78 (64)
B. multivorans	48	44 (63)	33 (58)	83 (90)	79 (83)	94 (94)	92 (94)	98 (96)	98 (94)
B. cenocepacia	80	35 (46)	30 (39)	55 (60)	51 (54)	66 (66)	63 (59)	74 (68)	66 (61)
B. cenocepacia III-A	50	32 (42)	26 (36)	50 (56)	46 (50)	58 (62)	54 (54)	68 (64)	60 (58)
B. cenocepacia III-B	30	40 (53)	37 (43)	63 (67)	60 (60)	80 (73)	77 (67)	83 (73)	77 (67)
B. stabilis	32	3 (9)	3 (9)	13 (25)	9 (9)	22 (31)	19 (19)	59 (47)	56 (34)
B. vietnamiensis	42	2 (17)	2 (12)	17 (38)	12 (26)	43 (55)	31 (48)	79 (64)	69 (62)
B. dolosa	18	28 (56)	17 (44)	78 (94)	78 (89)	94 (94)	94 (94)	100 (94)	94 (94)
B. ambifaria	11	45 (45)	45 (45)	73 (82)	73 (64)	100 (82)	91 (82)	100 (82)	100 (82)
B. anthina	13	92 (100)	85 (85)	92 (100)	92 (100)	100 (100)	100 (100)	100 (100)	100 (100)
B. pyrrocinia	2	50 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Key:
Pos, orange colouration in well positive indicative of carbohydrate oxidation; Neg, orange colouration not indicative of carbohydrate oxidation

(#): Percentages from duplicate runs

Referring to the oxidation of cellobiose in the 50CH strips, the results for Bcc strains originally referred to as genomovars I-V showed maximum correlation with these screening results after 72 hrs. However, a discrepancy was noted with *B. stabilis* strains, 100 % of strains oxidised cellobiose on the 50CH strips, but only a very small number oxidised the carbohydrate in the microtitre assay.

Table 3.14: Cumulative percentages of strains oxidising maltose

Bcc species	no.	48hr		72hr		96hr		5day	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
B. cepacia	36	8 (14)	6 (3)	36 (42)	22 (17)	53 (50)	33 (36)	67 (64)	47 (56)
B. multivorans	48	42 (38)	10 (21)	79 (79)	67 (75)	90 (90)	83 (90)	92 (96)	90 (94)
B. cenocepacia	80	25 (30)	15 (16)	54 (49)	44 (39)	59 (60)	51 (48)	60 (66)	48 (54)
B. cenocepacia III-A	50	22 (30)	12 (16)	52 (48)	42 (42)	54 (58)	50 (48)	56 (62)	42 (56)
B. cenocepacia III-B	30	30 (30)	20 (17)	57 (50)	47 (33)	67 (63)	53 (47)	67 (73)	57 (50)
B. stabilis	32	6 (9)	6 (6)	13 (28)	6 (13)	19 (34)	9 (22)	28 (34)	19 (34)
B. vietnamiensis	42	2 (5)	2 (5)	2 (12)	2 (7)	7 (24)	5 (14)	17 (36)	14 (31)
B. dolosa	18	22 (17)	11 (17)	67 (67)	56 (50)	78 (89)	78 (78)	83 (89)	83 (83)
B. ambifaria	11	9 (18)	0 (9)	9 (27)	9 (18)	45 (36)	9 (18)	64 (45)	45 (36)
B. anthina	13	54 (92)	15 (85)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
B. pyrrocinia	2	0 (0)	0 (0)	0 (50)	0 (50)	0 (50)	0 (50)	0 (50)	0 (50)

Key:
Pos, orange colouration in well positive indicative of carbohydrate oxidation; Neg, orange colouration not indicative of carbohydrate oxidation

(#): Percentages from duplicate runs

After 48 hrs, very few strains of *B. cepacia*, *B. stabilis*, *B. vietnamiensis*, *B. ambifaria*, and *B. pyrrocinia*, and a slightly higher percentage of all other Bcc species oxidised maltose, but to no great degree. Over the five day incubation period, as with cellobiose oxidation, the percentages of organisms oxidising maltose slowly rose. However, after 72 hrs, there were still very few strains of *B. stabilis*, *B. vietnamiensis*, *B. ambifaria* and

B. pyrrocinia which showed signs of oxidizing maltose, but 100 % of *B. anthina* oxidised the carbohydrate. After five days incubation, there were still only a few strains of *B. vietnamiensis* and *B. pyrrocinia* oxidising maltose. Also, in addition to all *B. pyrrocinia* strains showing indication of oxidation, the majority of *B. multivorans* strains oxidised the carbohydrate.

The results from the 50CH strips suggested that 100 % of *B. stabilis* oxidised maltose, compared to around 30 % of strains in the extended screen. The remaining Bcc species correlated quite well to the screening results, again, showing maximum correlation after 72 hrs incubation in extended screen.

Table 3.15: Cumulative percentages of strains oxidising trehalose

Bcc species	no.	48hr		72hr		96hr		5day	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
B. cepacia	36	3 (3)	3 (3)	8 (14)	8 (6)	28 (19)	17 (14)	33 (42)	31 (42)
B. multivorans	48	6 (4)	2 (4)	23 (31)	10 (13)	63 (58)	50 (54)	71 (88)	69 (88)
B. cenocepacia	80	4 (3)	1 (3)	28 (25)	18 (18)	45 (41)	43 (36)	55 (60)	54 (58)
B. cenocepacia III-A	50	2 (2)	2 (2)	24 (22)	14 (16)	40 (40)	40 (36)	52 (60)	50 (58)
B. cenocepacia III-B	30	7 (3)	0 (3)	33 (30)	23 (20)	53 (43)	47 (37)	60 (60)	60 (57)
B. stabilis	32	0 (3)	0 (3)	6 (6)	6 (6)	6 (16)	6 (13)	16 (31)	16 (28)
B. vietnamiensis	42	2 (0)	2 (0)	2 (0)	2 (0)	2 (2)	2 (2)	5 (12)	5 (7)
B. dolosa	17	0 (0)	0 (0)	6 (0)	6 (0)	12 (6)	12 (6)	12 (6)	12 (6)
B. ambifaria	11	0 (0)	0 (0)	0 (9)	0 (9)	9 (9)	9 (9)	18 (9)	9 (9)
B. anthina	13	0 (0)	0 (0)	0 (0)	0 (0)	15 (0)	0 (0)	15 (0)	0 (0)
B. pyrrocinia	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (50)	0 (50)

Key:
Pos, orange colouration in well positive indicative of carbohydrate oxidation; Neg, orange colouration not indicative of carbohydrate oxidation

(#): Percentages from duplicate runs

After 48 hrs, few strains showed evidence of trehalose oxidation (<7 %). Percentages of strains showing signs of oxidation again steadily rose over the five day incubation period. After 5 days, there were no Bcc species showing a high percentage of strains oxidising trehalose, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, *B. anthina* and *B. pyrrocinia* strains still showed little sign of trehalose oxidation. Referring to the oxidation of trehalose in the 50CH strips, the results for Bcc genomovars I-V showed maximum correlation with these screening results after 72 hrs. However, a discrepency was noted again with *B. stabilis* strains, with 100 % of strains oxidising trehalose on the 50CH strips, but only a very small number oxidised the carbohydrate in the screening exercise. It was found that if the observation of an orange colour was included as a positive oxidation reaction, either in the microtitre wells or in the 50CH strip (i.e. a colour change assigned a score of 1 or 2), this gave no improvement on discrimination.

Chromogenic strips

Appendix 3.11a contains the original scores assigned to all reactions recorded in the strips, including to those of the *E. coli* control strain. Appendix 3.11b lists the full names of all the indoxylic substrates used in these chromogenic strips.

There were no strains of Bcc and *P. aeruginosa* which produced enzymes to hydrolyse the substrates X ßD GLU, X ßD GUR, magenta ßD GUR, rose ßD GUR, blue ßD GUR, Y ßD GUR, X ßD fucoside, X sulphate, Magenta sulphate, Y sulphate, blue D ala and blue D leu.

Of the remaining substrates tested, a number gave good discrimination between Bcc and *P. aeruginosa* strains, the percentages of strains hydrolysing these substrates are shown in Table 3.16. All Bcc strains produced phosphatase that hydrolysed XP, compared to only 31 % of *P. aeruginosa* strains. The table shows that of the remaining substrates, hydrolysis of which could possibly discriminate between these two groups of organisms, none was hydrolysed by *P. aeruginosa* compared to at least 77 % of Bcc hydrolysing the substrates. Of particular discriminatory significance were the substrates Rose P and YP, 100 % of Bcc strains hydrolysed these substrates.

Table 3.17 shows the results for those substrates, hydrolysis of which demonstrated potential for discrimination between Bcc genomovars. These substrates were not hydrolysed by any *P. aeruginosa* strains except Y ßD GAL, which was hydrolysed by 8 % of strains, rose caprylate (38 % of strains), and blue L ala (54 % of strains). The number of strains used in this study were small and therefore the results are merely an indication of potential discriminatory power of these chromogenic substrates.

3.16: Cumulative percentages of Bcc and *P. aeruginosa* strains hydrolysing indoxylic substrates on chromogenic strips

Organism	No. of strains	X NAGlu	Magenta NAGlu	Rose NAGlu	F ßD GAL	Y ßD GAL	Magenta P	Rose P	Y P
Bcc	**13**	77	77	77	85	85	92	100	100
P. aeruginosa	**13**	0	0	0	0	8	0	0	0

		Magenta ßD GLU	Rose ßD GLU	8 HQ ßDGLU	Y ßD GLU	X ßD cellobioside	Rose ßD cellobioside	X ßD Xyl
Bcc	**13**	92	92	69	77	85	77	69
P. aeruginosa	**13**	0	0	0	0	0	0	0

3.17: Cumulative percentages of Bcc species hydrolysing indoxylic substrates on Chromogenic strips

Organism	No.	X NAGlu	Magenta NAGlu	Rose NAGlu	X NAGal	X ßD GAL	Magenta ß GAL	Rose ßD GAL	Blue ßD GAL	4 Cl 3 I ßDGAl	F ßD GAL	Magenta P
Bcc	13	77	77	77	38	15	15	31	23	23	85	92
B. cepacia	2	100	100	100	50	0	0	0	0	0	100	100
B. multivorans	2	100	100	100	0	0	0	100	50	0	100	100
B. cenocepacia	2	100	100	100	100	0	0	0	0	0	100	100
B. stabilis	2	50	50	50	0	0	0	0	0	50	50	50
B. vietnamiensis	2	0	0	0	0	0	0	0	0	0	50	100
B. dolosa	2	100	100	100	50	50	50	50	50	50	100	100
B. ambifaria	1	100	100	100	100	100	100	100	100	100	100	100

		Magenta ßD GLU	Rose ßD GLU	8 HQ ßDGLU	Y ßD GLU	X ßD cello	Rose ßD cello	X αD GLU	X αD Mann	Rose αD Mann	Blue αD Mann	Rose caprylate
Bcc	13	92	92	69	77	85	77	46	54	54	8	77
B. cepacia	2	100	100	100	100	100	100	50	100	100	50	100
B. multivorans	2	100	100	100	100	100	100	50	100	100	0	100
B. cenocepacia	2	100	100	100	100	100	100	100	100	100	0	100
B. stabilis	2	50	50	50	50	50	50	0	50	50	0	100
B. vietnamiensis	2	100	100	50	0	50	0	0	0	0	0	50
B. dolosa	2	100	100	0	100	100	100	50	0	0	0	50
B. ambifaria	1	100	100	100	100	100	100	100	0	0	0	0

		8 HQ ßD GAL	Y ßD GAL	5 I 3 I ßD GAL	Green ßD GAL	X αD GAL	Magenta αD GAl	Rose αD GAL	Blue αD GAL	X ßD Xyl	X ßD fucoside	Blue L ala
Bcc	13	8	85	8	15	31	23	15	15	69	15	62
B. cepacia	2	0	100	0	0	50	50	50	50	100	0	100
B. multivorans	2	0	100	0	0	0	0	0	0	100	0	100
B. cenocepacia	2	0	100	0	0	0	0	0	0	100	0	100
B. stabilis	2	0	50	0	0	50	50	50	50	0	0	100
B. vietnamiensis	2	0	50	0	0	0	0	0	0	50	50	0
B. dolosa	2	50	100	50	50	50	0	0	0	50	0	0
B. ambifaria	1	0	100	0	100	100	100	0	0	100	100	0

Of the numerous substrates showing potential for Bcc species differentiation (genomovars I to VII), the most promising ones were XNAGal, mag NAGlu and rose NAGlu, hydrolysis of which could discriminate *B. stabilis* or *B. vietnamiensis* strains from the other Bcc species. Hydrolysis of XßDGAL, magenta ß GAL, and green ßD GAL could identify a strain as *B. dolosa* or *B. ambifaria.* The substrate blue L ala was hydrolysed by 100% of *B. cepacia, B. multivorans, B. cenocepacia,* and *B. stabilis* strains but by no strains of *B. vietnamiensis, B. dolosa* or *B. ambifaria. B. dolosa* was the only strain not to hydrolyse 8HQ ßD GAL and the only strain to hydrolyse 5 I 3 I ßD GAL. X αD Mann and rose αD Mann were hydrolysed by all *B. cepacia, B. multivorans* and *B. cenocepacia* strains, but by no strains of *B. vietnamiensis, B. dolosa* and *B. ambifaria.* All Bcc strains hydrolysed rose ßD GLU and mag ßD GLU except one strain of *B. stabilis.* The only organism to hydrolyse blue αD mann was one strain of *B. cepacia.* All Bcc strains hydrolysed magenta P except one strain of *B. stabilis.*

The remaining substrates which were in the strips i.e. XP, X acetate, Y acetate, X butyrate, X caprylate and magenta caprylate were hydrolysed by all Bcc strains so showed no differentiation between the different Bcc species, were also hydrolysed by a significant percentage of *P. aeruginosa* strains so hydrolysis of these substrates could not differentiate between Bcc and *P. aeruginosa* strains.

Discussion

Since the recognition of "*B. cepacia*" as a major pathogen in patients with CF in the late 1970s and early 1980s (Isles *et al.*, 1984; Thomassen and Demko, 1985), the identification of this organism has been the subject of ongoing research, in particular more recently with regard to differentiation of the species comprising the Bcc. The urgent need for a detailed understanding of the clinical risks posed by each Bcc species stresses the necessity for accurate methods for differentiating these strains. Incomplete or incorrect identification of an organism as Bcc can lead to inappropriate segregation or cohorting of CF patients. Infection control is a significant problem associated with members of the Bcc and there have been extensive studies of its molecular epidemiology (Martone *et al.*, 1987; Kostman *et al.*, 1992; Fisher *et al.*, 1993; Mahenthiralingam *et al.*, 1997; Agodi *et al.*, 2001; Coenye *et al.*, 2002; Petrucca *et al.*, 2003).

Although there are a number of successful molecular techniques which can confidently identify the species comprising the Bcc, there is no such reliable methodology for phenotypic identification. There is much information available on the phenotypic characteristics of Bcc strains, however, most of these studies date back before the unraveling of its complex taxonomy (Welch *et al.*, 1987; Carson *et al.*, 1988; Kämpfer and Dott, 1988; McKevitt *et al.*, 1989; Wauters *et al.*, 1995).

Several studies have indicated problems with discrimination between the individual Bcc species using phenotypic methods or commercial bacterial identification systems (Kiska *et al.*, 1996; Henry *et al.*, 1997, Coenye *et al*, 2000, McMenamin *et al.*, 2000).

Identification of *Burkholderia* spp., *Pandoraea* spp., *R. pickettii*, *B. gladioli*, or *Alcaligenes* spp. by commercial methods such as the API 20NE, Remel, RapID NF Plus, Microscan, Crystal, Sherlock, and Vitek tests lacks accuracy (Kiska *et al.*, 1996; Van Pelt *et al.*, 1999; Shelly *et al.*, 2000; Brisse *et al.*, 2002), and results must be confirmed by other methods. Shelly *et al.*, (2000) reported positive predictive values between 71 % and 98 % for these systems when they were used as the primary identification method. They reported that there was at least a 20 % probability of a strain identified by these tests as non-"*B. cepacia*" actually being a member of the Bcc. Ideally commercial systems should be supplemented with additional biochemical tests, but current phenotypic methods alone should not be relied upon for definitive identification. The general conclusion to date is that organisms suspected of being of the Bcc be subjected to a combination of biochemical tests and molecular diagnostic assays for species identification (Van Pelt *et al.*, 1999; McMenamin *et al.*, 2000; Henry *et al.*, 2001). However, such techniques are not always suitable for routine diagnostic microbiology laboratories, as many do not possess the necessary expensive equipment required for genotypic analysis. A reliable identification system for Bcc species based on phenotypic tests alone is therefore desirable.

Little is currently known of the enzyme profiles of the nine Bcc species. Despite the wide array of substrates and enzyme tests available, in general, few are strongly associated with an individual target species. This study confirms this also applies to the Bcc species. To optimise the identification of bacteria, many systems use a combination of two or more substrates with different end colours or fluorescence, leading to a more accurate and sensitive identification of target species. For example, a rapid 30 minute process for the identification of *Streptococcus bovis* has been suggested, which uses

three enzyme substrates in broth format: *p*NP-α-galactoside and MU-ß-glucoside in combination in one tube and pyrrolidonyl-ß-naphthylamide in a second tube, to differentiate *S. bovis* from other streptococci and enterococci (Panosian and Edberg 1989).

Discrimination of Bcc strains from closely related organisms

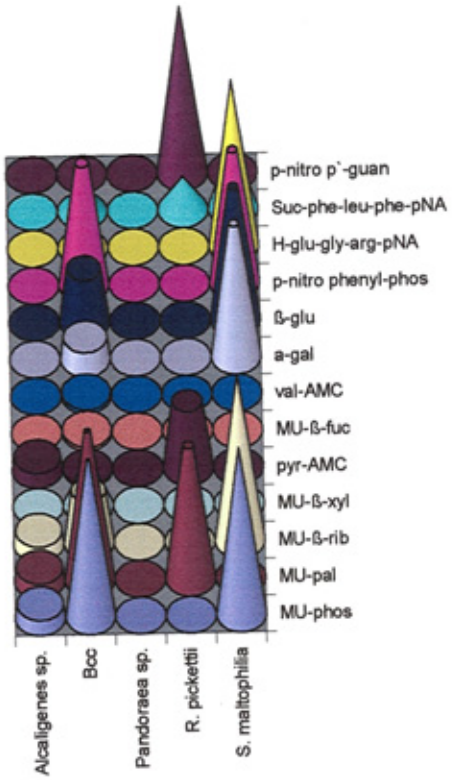
Prior to looking at discrimination between the different species comprising the Bcc, an isolate must first be identified as being a member of the Bcc, i.e. discriminated from closely related organisms. Figure 3.12 illustrates the relative hydrolyses of those enzyme substrates screened against organism collection B, which showed potential for such discrimination.

Coenye *et al.* (2000), Henry *et al.* (2001) and Moore *et al.* (2002b) described the particular problem of *Pandoraea* spp. identification. This genus is not included in commercial kits such as the API 20 NE identification system database and would most likely give identification of *Alcaligenes* spp., at low discrimination, or an unacceptable identification (Henry *et al.*, 2001). Investigation into the enzyme profiles of these organisms revealed that members of the genus *Pandoraea* were unreactive with a number of substrates although *Pandoraea spp.* and *Ralstonia spp.* were among the only strains to produce DL-leucine carboxypeptidase. Taking into consideration the discrimination potential of the substrates and reproducibility of the tests, the most useful enzyme substrates found for identification of *Pandoraea* spp. were MU-palmitate, MU-phosphate and *p*NP-myristate, as the majority of *Pandoraea* spp. tested did not produce

the enzymes palmitate esterase, phosphatase or myristate esterase but these enzymes were produced by the majority of other strains of species closely related to Bcc.

In combination with hydrolysis of MU-phosphate in particular, this test could improve current identification of *Pandoraea* sp. Henry *et al.* (2001) found that simple phenotypic tests were also extremely poor at identifying *R. pickettii* although they did establish that a combination of the oxidation of maltose, lactose, xylose and adonitol, and the oxidase reaction were useful to distinguish *B. gladioli*, *R. pickettii* and *Pandoraea sp* from Bcc strains. Hydrolysis of benzoyl-L-glutamic acid was shown to be a useful test for identification of *R. pickettii*. However, the most discriminatory test for this organism was hydrolysis of *p*NP-p′-guanidinobenzoate, a substrate for α and ß-trypsin. Other than a low percentage of uncommonly isolated *Ralstonia* sp., there were no organisms that produced this enzyme apart from *R. pickettii*. Hydrolysis of MU-palmitate showed potential for differentiating between *R. pickettii* and other *Ralstonia* sp. A number of substrates could differentiate between *Pandoraea* sp. and *Ralstonia* sp. *Ralstonia* spp. produced glutamic carboxypeptidase, histidine carboxypeptidase, palmitate esterase and α and ß-trypsin, *Pandoraea* spp. were found to produce none of these enzymes.

Figure 3.12: Chart showing relative hydrolyses of those enzyme substrates which gave potential discrimination between Bcc isolates and closely related organisms



Key:
MU-phos, 4-methylumbelliferyl phosphate; MU-pal, 4-methylumbelliferyl palmitate; MU-ß-rib, 4-methylumbelliferyl-ß-riboside; MU-ß-xyl, 4-methylumbelliferyl-ß-xyloside; pyr-AMC, pyroglutamyl-7-amido-4-methylcoumarin; MU-ß-fuc, 4-methylumbelliferyl-ß-fucoside; val-AMC, valyl-7-amido-4-methylcoumarin; α-gal, 4-methylumbelliferyl-α-D-galactoside; ß-glu, 4-methylumbelliferyl-ß-glucoside; *p*-nitro phen-phos, *p*-nitrophenyl phenyl phosphonate; *p*-nitro p`-guan, *p*-nitrophenyl p`-guanidinobenzoate

Bcc strains produced a number of enzymes that *P. aeruginosa* strains did not. These include alanine carboxypeptidase, phenylalanine carboxypeptidase, DL-methionine carboxypeptidase and phosphatases. Figure 3.12 illustrates the high proportion of Bcc strains hydrolyzing MU-phosphate, MU-palmitate and p-NP phenyl phosphonate compared to many of the closely related organisms.

Kämpfer and Dott, (1988) found that *Alcaligenes* sp. and *Acinetobacter* sp. could be differentiated from "*B. cepacia*" due to the production of N-acetyl-ß-D-glucosaminidase (NAGase), phosphatase, and phospholipase C (hydrolysis of *p*NP-phosphoryl choline) in "*B. cepacia*" strains. The results of the present study confirm this, as *Acinetobacter* sp. were found not to produce NAGase or phosphatase, and *Alcaligenes* sp. were found not to produce phosphatase or hydrolyse phosphoryl choline. Perry *et al.* (1998) determined that a high percentage of *Acinetobacter* sp., in particular *A. baumanii* and *A. calcoaceticus*, produced a number of carboxypeptidases, including all those screened for in the current study. The present study confirmed alanine, glycine, histidine and DL-methionine carboxypeptidases to be commonly produced by these species. In general though, it was found that *Acinetobacter* spp., like *Pandoraea* spp., produced very few enzymes that were screened for in this study compared to other species.

H-glu-gly-arg-*p*NA, a sensitive substrate for urokinase, was an excellent substrate for the identification of *S. maltophilia*, with few other strains screened producing this endopeptidase. The clinical importance of this opportunistic pathogen is well known. It is responsible for causing infections in debilitated patients with impaired host defence mechanisms, including pneumonia, meningitis and wound infections. As *S. maltophilia* is resistant to most commonly used ß-lactam and aminoglycoside antibiotics, patients receiving long-term antibiotic therapy are particularly at risk for acquiring infections with this organism (Murray *et al.*, 1998). Problems with accurate identification of *S. maltophilia* have previously been documented (Saiman *et al.*, 2001; Ferroni *et al.*, 2003). A rapid accurate test for *S. maltophilia* confirmation could therefore be of clinical interest. Screening of a more extensive collection of *S. maltophilia* strains for

urokinase production using this substrate is warranted to determine whether development of such a test may be possible using H-glu-gly-arg-*p*NA.

As Table 3.16 shows, significant discrimination was found between abilities of Bcc and *P. aeruginosa* strains to hydrolyse the indoxylic substrates within the chromogenic strips from bioMérieux. However, no firm conclusions can be drawn as a limited number of strains were used. The object of this exercise was merely to assess the potential for the application of such chromogenic substrates in solid media for Bcc species identification.

Discrimination between Bcc species.

Once an isolate has been accurately identified as being a member of the Bcc, the Bcc species allocation must then be determined. This becomes more difficult as the number of species found to belong to the complex continues to increase (Mahenthiralingam *et al.*, 2000a, 2002; Vandamme *et al.*, 2000, 2002, 2003; Coenye *et al.*, 2001b, 2001c). The enzyme profile screening carried out in this current study was performed on those genomovars or species formally identified and available at the time.

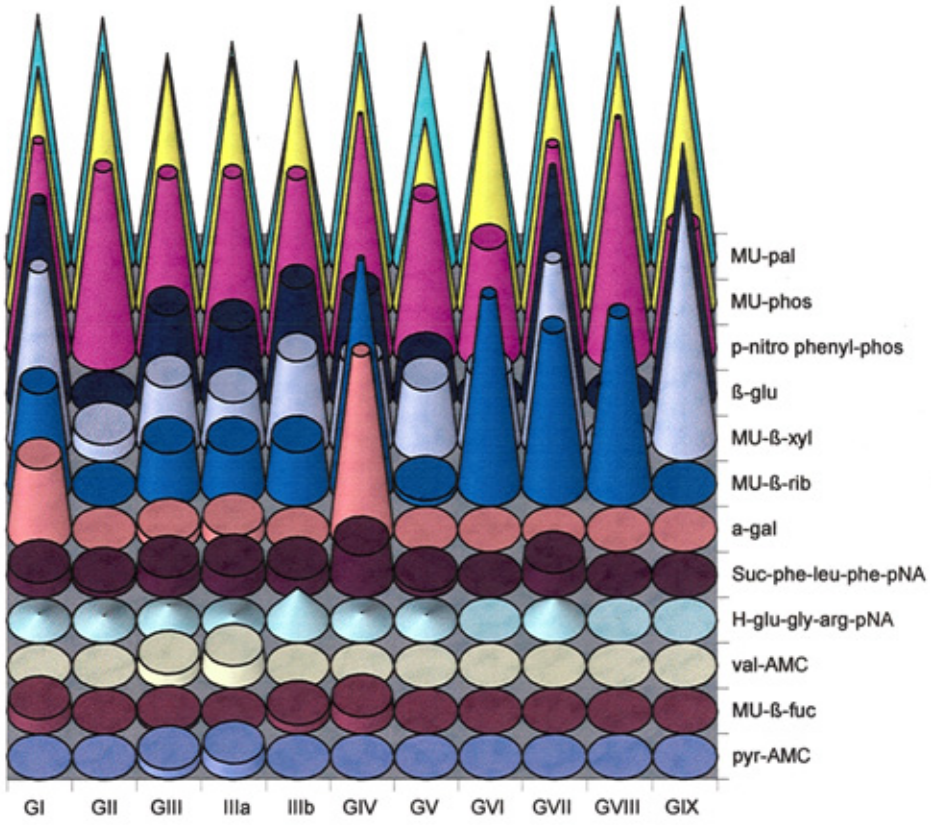
The accuracy with which organisms classified as belonging to the Bcc, both as a group, and the individual species comprising the Bcc, are identified in the laboratory determines therapeutic options and infection control measures (McMenamin *et al.*, 2000). Incomplete or incorrect identification of an organism can lead to inappropriate segregation of patients with CF, as the only effective method of infection control is to physically separate Bcc-infected patients from non-infected patients (Whitby *et al.*, 2000b). Preliminary studies indicate that strains associated with transmissiblity and

increased morbidity and mortality, cluster in *B. cenocepacia* (LiPuma *et al.*, 1999, 2001; Speert *et al.*, 2002; Clode *et al.*, 2000). Patients infected pre-transplant with *B. cenocepacia* as opposed to other Bcc species are at highest risk of Bcc-related mortality (Aris *et al.*, 2001; De Soyza *et al.*, 2001). Thus, accurate identification of the Bcc species is essential for effective patient management.

Henry *et al.* (2001) and Vermis *et al.* (2003) concluded that phenotypic characteristics are not sufficient for differentiating all nine species comprising the Bcc. As Figure 3.13 illustrates, hydrolysis of the enzyme substrates tested in this study could also not definitively differentiate the nine Bcc species. However, our present findings have added a wealth of phenotypic information to that already known of the individual Bcc species and may be useful for the development of phenotypic identification schemes in the future.

A number of the substrates tested were not cleaved by any of the Bcc species and were therefore found to be of no discriminative value. These results are in keeping with those of Kämpfer and Dott, (1988) who found that none of ten strains of "*B. cepacia*" screened produced the following enzymes: ß-glucuronidase, α-mannosidase, L-pyroglutamic acid aminopeptidase, chymotrypsin and valine aminopeptidase. However, this working group also found the "*B. cepacia*" strains did not produce ß-xylosidase, whereas our study revealed a large percentage of Bcc strains produced this enzyme. Perry *et al.* (1998) found that no Bcc strains produced L-glutamic acid carboxypeptidase or DL-leucine carboxypeptidase, our study also found very low numbers of strains to produce these enzymes. In contrast to their findings of no Bcc strains producing L-histidine carboxypeptidase, we found 51-74 % of strains produced this enzyme.

Figure 3.13: Chart showing relative hydrolyses of those enzyme substrates which gave potential discrimination between the individual species of the Bcc



Key:
MU-pal, 4-methylumbelliferyl palmitate; MU-phos, 4-methylumbelliferyl phosphate; *p*-nitro phenyl-phos, *p*-nitrophenyl phenyl phosphonate; ß-glu, 4-methylumbelliferyl-ß-glucoside; MU-ß-xyl, 4-methylumbelliferyl-ß-xyloside; MU-ß-rib, 4-methylumbelliferyl-ß-riboside; α-gal, 4-methylumbelliferyl-α-D-galactoside; val-AMC, valyl-7-amido-4-methylcoumarin; MU-ß-fuc, 4-methylumbelliferyl-ß-fucoside; pyr-AMC, pyroglutamyl-7-amido-4-methylcoumarin

Henry *et al.*, (2001) found that a combination of the oxidation of sucrose, maltose, lactose, xylose and adonitol, ONPG utilisation, growth at 42 ^{O}C and the oxidase reaction were useful to differentiate *B. multivorans*, *B. stabilis*, *B. vietnamiensis* ~~and *B. dolosa*~~ from each other but found that phenotypic similarity between *B. multivorans* and *B. dolosa* is high, except for the characteristic earthy, rotten potato odor associated with

the latter genomovar. Vermis *et al*, (2003) found that Bcc isolates are heterogenous in the utilisation of carbon sources, *B. dolosa* was found to be the only species which could be clearly differentiated from the other Bcc species based on its substantially different carbon source assimilation pattern coupled with its failure to grow on PCAT medium. In contrast to this we found that *B. dolosa* could not be differentiated using enzyme profiles, or through the oxidation of cellobiose, maltose or trehalose. We also found phenotypic similarity between *B. multivorans* and *B. dolosa* also to be high, although differences were observed in their ß-ribosidase production and trehalose oxidation. Welch *et al.*, (1987) found that of 26 isolates of Bcc, 100 % oxidised glucose, lactose, maltose, mannitol, and 97 % oxidised xylose, so as a result of such high values, it can be confidently assumed that this study also found no discrimination between Bcc species.

Henry *et al.* (2001) concluded that *B. cepacia*, *B. cenocepacia* and *B. ambifaria* remained very difficult to differentiate by biochemical testing. There were no enzyme substrates identified in this current study which could definitively differentiate between these three species. However, differences were observed in cellobiose oxidation and MU-α-galactoside hydrolysis in *B. cepacia*, compared to the other two. Oxidation of a number of the carbohydrates in the 50CH strips may be of discriminative value between Bcc species, including potential discrimination between *B. cepacia* and *B. cenocepacia*, for example, glycerol, arbutin and amygdalin. However, as it proved not possible to reproduce the results from the strips in microtitre format for these particular carbohydrates, further work is required to assess these carbohydrates with larger numbers of isolates.

Biochemical differences between the two *recA* subgroups of *B. cenocepacia* screened (III-A and III-B) were not observed by Henry *et al*, (2001) except for the ability to reduce nitrate, which was never observed for *B. cenocepacia* subgroup III-B but was variable among III-A strains. There were no differences observed in the ability of these two subgroups to hydrolyse any of the enzyme substrates tested in this study (see Figure 3.13) or in cellobiose, maltose or trehalose oxidation, confirming their phenotypic similarity.

Vermis *et al.* (2003) found no discrimination of *B. anthina* and/or *B. pyrrocinia* with various carbon source utilisation, antibiotic susceptibility and growth ability on selective media. Although only low numbers were available for our study, particularly of *B. pyrrocinia* (n=2), the results provide a basis on which to follow up these findings with further studies. *B. anthina* could be differentiated from *B. cepacia, B. stabilis, B. vietnamiensis* and *B. ambifaria*, using MU-ß-glucoside and MU-ß-xyloside hydrolysis, and cellobiose oxidation. *B. pyrrocinia* could be differentiated from *B. multivorans, B. stabilis, B. dolosa* and *B. anthina* by MU-ß-riboside, MU-ß-xyloside and MU-ß-glucoside hydrolysis and also trehalose oxidation. The two strains of *B. anthina* did not oxidise cellobiose, whereas at least some percentage of all other Bcc species did, although some had variable reactions.

For the endopeptidase substrates, there was little correlation observed between hydrolysis of substrates specific for particular endopeptidases, and the production of an endopeptidase from a particular organism. For example, an organism could hydrolyse one substrate specific for chymotrypsin, but not a second substrate also specific for chymotrypsin. The substrates which were hydrolysed by Bcc strains gave no

discrimination between species, and the hydrolysing endopeptidases were produced by a percentage of all nine Bcc species. These endopeptidases include cathepsin G, proteinase yscE, chymotrypsin, tripeptidyl peptidase I, cystyl aminopeptidase (oxytocinase), human and rat leukocyte and porcine pancreatic elastases, proteinase K, subtilisins and thermitase, macrophilins, cyclophilins and bifunctional glutathionylspermidine (GSP).

Advantages/disadvantages of using enzymes for bacterial identification

The use of enzymes as taxonomic indicators has certain potential advantages over several other methods. These include ease of performance, flexibility for use in a variety of situations e.g. liquid media, agar based plates, microtitre wells, applicability to diverse organisms such as aerobic/anaerobic, fast/slow growing, and lack of necessity for complex instrumentation or for expensive and purified antisera (James, 1994). The trend in microbial identification is to decrease analysis time while maintaining or improving one's ability to identify the unknown microorganism. Identification based on microbial enzyme activity profiles provides the basis for a truly rapid system. Enzyme substrates can also be highly sensitive and specific at low concentrations (Manafi *et al.*, 1991). An important feature of fluorescence emission is the sensitivity of detection or measurement. With highly fluorescent molecules such as fluorescein, 4-methylumbelliferone or 7-amino-4-methylcoumarin the quantum yield is high and approaches unity under optimal conditions. A wide range of substrates can be assayed simultaneously, providing a detailed biochemical profile of the organism, which can then be identified using a database. Also, as these tests are used to detect the presence of

pre-formed enzymes, they can aid in the identification of more fastidious organisms such as anaerobes, for which more conventional testing may take longer periods of time.

The substrate chosen is dependent upon the application for which detection of the enzyme production is sought. For example, *p*NP-ß-glucuronide is suitable for liquid media, but less suitable for solid media because of the extensive diffusion of the yellow colour of the released nitrophenol (Delisle *et al.*, 1989). Substrates for selected enzymatic activities based on such core molecules as nitrophenols, *p*-nitroanilides and indoxyls can be directly loaded in suitably buffered solution into a series of tubes, inoculated and incubated for a set period of time at optimal temperatures. The appearance of colour is automatic in these cases so that time can be chosen to give the best differentiation of positive and negative cultures. Naphthol- or naphthylamine-based substrates may be detected by addition of azo-coupling reagents such as Fast Blue RR salt or alternatively by addition of 4-dimethylaminocinnamaldehyde, which yields a purple-red Schiff's base with the released amine.

MU-glucoside substrates can be incorporated into liquid or solid media. Because the fluorescence of MU is pH dependent, the pH of growth media containing this substrate should be neutral or slightly alkaline, otherwise alkaline solution must be added to reveal the fluorescence (Freier *et al.*, 1987). MU-glucoside can be sterilized together with other medium ingredients without loss of activity.

However, there are drawbacks with these systems. For example, only pure strains of bacteria can be used, as two or more strains in combination could form the profile of an entirely different strain, thereby giving a false result. Furthermore, the test kits are

relatively expensive, due to the presence of many chromogenic substrates and reagents, and are therefore only generally used to confirm the identity of unknown species. The method is limited by enzyme availability to the substrates, substrate turnover rate, and the sensitivity of the detection system (James, 1994), all of which however, can be addressed experimentally. Fluorogenic compounds are relatively unstable. Some compounds are relatively water insoluble and consequently require initial dissolution in a small volume of a high dielectric constant solvent such as dimethyl sulphoxide (DMSO). Dilution with buffer may then yield a homogeneous solution. This is a particular problem with long-chain esters of 4-methylumbelliferone and with some endopeptidase substrates due to the lipophilic nature of the N-terminal blocking group (Brynes *et al.* 1981).

Product detection

In the detection of specific enzymes, visualisation of the substrate-product transformation is central. While selectivity of the procedure resides essentially in correct choice of substrate and conditions of the test, the sensitivity of the method is largely governed by the nature of the substrate and the method of visualisation used. Broadly speaking, two major methods are employed (James, 1994). Visual observation and colorimetric analysis, on the one hand, are convenient procedures for routine characterisation and identification, as used in this study with the *p*NA-, *p*NP-, and benzoyl-linked substrates. On the other hand, fluorescence observation and fluorimetric measurement are useful procedures, being both rapid and sensitive, thereby permitting the use of smaller inocula and allowing speciation or identification of relatively slow

growing organisms such as mycobacteria (Grange and Clark, 1977) as used in this study with the MU- and 7AMC-linked substrates.

Since the cost of many substrates employed is quite high, the screening performed in this study shows that microtitre plates may successfully be used to accommodate the tests. As well as adding the substrate in solution to the wells, substrates may alternatively be dried into the wells and subsequently reconstituted with buffer prior to incubation. Where a quantitative assessment is required or a numerical value generated, the use of microtitre plates read by an ELISA reader has a potential applications (James, 1994). The use of strip tests based on selected chromogenic substrates simplifies the procedure considerably. A number of API strips for diagnostic use are commercially available and also many more for investigational purposes (bioMérieux, Marcy L`Etoile, France). In particular, the API-ZYM method has been widely applied (Slots, 1981, Laughon *et al.*, 1982).

Chromogenic media

Rapid detection and identification of microorganisms is of high importance in a diverse array of applied and research fields. The ability to detect the presence of a specific and exclusive enzyme using suitable substrates, in particular fluorogenic or chromogenic enzyme substrates, has led to the development of a great number of methods for the identification of microorganisms involving primary isolation media (Wright, 1984; Brenner *et al.*, 1993; Manafi, 1996; 1998; Perry *et al.*, 1999). By incorporation of synthetic enzyme substrates into primary isolation media, enumeration and detection can be performed directly on the isolation plate and the need for subculture and further

biochemical tests to establish the identity of certain microorganisms is reduced or even eliminated (Manafi *et al.*, 1991; Manafi, 1996).

The chromogenic enzyme substrates used in this study for the large-scale screening of the Bcc and closely related organism collections (i.e. *p*NP- and *p*NA-linked substrates) are soluble in water and as a result are widely used in liquid assays, such as multi-well assays. However, they are less suitable for agar-based culture as the coloured product diffuses away from positive colonies, making identification difficult. (Le Minor and Ben Hamida, 1962; Bürger, 1967),

Non-diffusible substrates

It is essential for a chromogenic substrate used in isolation media to be non-diffusable in order to allow specific colony identification. The most commonly used non-diffusible chromogenic substrates are derivatives of the indoxyl group. Indoxyls are very adaptable and small modifications to the chemical structure can change the resulting colour of the compound. For example, 5-bromo-4-chloro-3-indoxyl forms a blue-green compound, 5-bromo-3-indoxyl forms a blue compound and 6-chloro-3-indoxyl forms a rose colour (Haughland, 1996). Halogenated derivatives of indoxyl are generally preferred because they produce finer precipitates and are therefore less likely to diffuse from the site of formation, i.e. the bacterial cell (Haughland, 1996).

The substrates incorporated into the Chromogenic strips from bioMérieux are all derivatives of the indoxyl group and could therefore potentially be applied in isolation media without diffusion of the product. Vermis *et al.* (2003) looked at growth ability on

selective media of Bcc isolates belonging to all nine species. Among the isolation media tested, BCSA and Mast *Burkholderia cepacia* medium were the most efficient for growth of all nine species. However, the lack of selectivity of these existing media is a drawback for their application to clinical and environmental samples. Our present findings may be useful for the development of improved isolation media.

Of the indoxyl substrates in the strips, for which hydrolysis of the equivalent fluorogenic substrate was also investigated, the results correlated very well, as would be expected as both substrates should be hydrolysed by the same enzyme. Table 3.18 shows the percentages of chromogenic substrates hydrolysed in the chromogenic strips, compared to the percentages of the equivalent fluorogenic substrates hydrolysed. The results in the table are compiled from the percentages of strains that hydrolysed each substrate in the strip, and an average percentage of strains which hydrolysed the fluorogenic substrates. The results for ß-glucuronidase and sulphatase are not shown, as all substrates tested which would be hydrolysed by these enzymes, both in the chromogenic strips and the fluorogenic screening, were not hydrolysed by any Bcc or *P. aeruginosa* strains.

The table illustrates the high correlation observed. For example, three substrates on the chromogenic strips linked to N-acetyl-ß-D-glucosaminide were hydrolysed by 77 % Bcc and 0 % *P. aeruginosa* strains. The equivalent fluorogenic substrate, MU-N-acetyl-ß-D-glucosaminide was hydrolysed by 68 % Bcc and 2 % of *P. aeruginosa* strains. Four indoxyl substrates in the strip detected phosphatase, all were hydrolysed by a high percentage of Bcc strains, and a low number of *P. aeruginosa* strains, this was also found with hydrolysis of MU-phosphate.

Table 3.18: Percentages of Bcc and *P. aeruginosa* strains hydrolysing chromogenic indoxyl substrates compared to the fluorogenic substrate equivalents

Enzyme	Substrate in C strip	Bcc	*P. aeruginosa*
ß-glucosaminidase	X NAGlu	77	0
ß-glucosaminidase	Magenta NAGlu	77	0
ß-glucosaminidase	Rose NAGlu	77	0
average fluorogenic screen results		**68**	**2**
ß-galactosidase	X ßD GAL	15	0
ß-galactosidase	Magenta ß GAL	15	0
ß-galactosidase	Rose ßD GAL	31	0
ß-galactosidase	Blue ßD GAL	23	0
ß-galactosidase	4 Cl 3 I ßDGAL	23	0
ß-galactosidase	F ßD GAL	85	0
ß-galactosidase	8 HQ ßD GAL	8	0
ß-galactosidase	Y ßD GAL	85	8
ß-galactosidase	5 I 3 I ßD GAL	8	0
ß-galactosidase	Green ßD GAL	15	0
average fluorogenic screen results		**3**	**0**
α-galactosidase	X αD GAL	31	0
α-galactosidase	Magenta αD GAL	23	0
α-galactosidase	Rose αD GAL	15	0
α-galactosidase	Blue αD GAL	15	0
average fluorogenic screen results		**17**	**0**
ß-glucosidase	X ßD GLU	0	0
ß-glucosidase	Magenta ßD GLU	92	0
ß-glucosidase	Rose ßD GLU	92	0
ß-glucosidase	8 HQ ßDGLU	69	0
ß-glucosidase	Y ßD GLU	77	0
average fluorogenic screen results		**48**	**9**
ß-cellobiosidase	X ßD cellobioside	85	0
ß-cellobiosidase	Rose ßD cellobioside	77	0
average fluorogenic screen results		**21**	**0**
α-glucosidase	X αD GLU	46	0
average fluorogenic screen results		**3**	**1**
α-mannosidase	X αD Mann	54	0
α-mannosidase	Rose αD Mann	54	0
α-mannosidase	Blue αD Mann	8	0
average fluorogenic screen results		**0**	**0**

Table 3.18 (cont`d..): Percentages of Bcc and *P. aeruginosa* strains hydrolysing chromogenic indoxyl substrates compared to the fluorogenic substrate equivalents

enzyme	substrate in strip	Bcc	*P. aeruginosa*
ß-xylosidase	X ßD Xyl	69	0
average fluorogenic screen results		**65.5**	**0.5**
ß-fucosidase	X ßD fucoside	15	0
ß-fucosidase	X ßL fucoside	0	0
average fluorogenic screen results		**22**	**0.5**
phosphatase	X P	100	31
phosphatase	Magenta P	92	0
phosphatase	Rose P	100	0
phosphatase	Y P	100	0
average fluorogenic screen results		**97.5**	**7**
acetate esterase	X acetate	100	77
acetate esterase	Y acetate	100	46
average fluorogenic screen results		**100**	**100**
butyrate esterase	X butyrate	100	85
average fluorogenic screen results		**100**	**100**
alanine aminopeptidase	Blue L ala	62	54
average fluorogenic screen results		**100**	**99**
leucine aminopeptidase	Blue L leu	0	0
average fluorogenic screen results		**89**	**99**

Although overall, correlation was good, there were a few substrates that did not correlate well. For example MU-α-glucoside was hydrolysed by very few Bcc strains but half of the Bcc strains tested hydrolysed the equivalent substrate on the chromogenic strips. However, both tests showed *P. aeruginosa* strains did not produce the enzyme α-glucosidase. Two out of the three α-mannosidase substrates on the chromogenic strips were hydrolysed by a much higher percentage of Bcc strains than the fluorogenic equivalents, although the third substrate (blue-α-D-mannoside) did correlate well. The reasons for these discrepancies are unknown. It could be speculated

that some strains are unable to take up either the chromogenic or fluorogenic substrate the cell, due to differences in the molecules.

As the majority of the indoxylic and fluorogenic substrate results correlated well, it would seem appropriate to use such large screening fluorogen-based screening exercises as a guide to explore potentially useful chromogenic substrates for application in isolation media. Figure 3.14 shows a strain of *B. cepacia* hydrolysing the substrate XNAG to produce green colonies. As the majority of these indoxylic substrates showed potential for the discrimination of Bcc strains from *P. aeruginosa*, in particular rose phosphate, this could be of significance if a chromogenic medium was desired which could discriminate between these two organisms.

Figure 3.14: *B. cepacia* (ATCC 25416) colonies growing on media containing XNAGlu (hydrolysed by N-acetyl-ß-glucosaminidase)

Ideally, a medium would be designed in which the species comprising the Bcc would hydrolyse various substrates to produce different coloured colonies on a plate, as in the simultaneous detection of *E.coli* and coliforms (Manafi, 2000). Commercially available media have been developed which permit rapid simultaneous detection of *E. coli* and coliforms in water. These media contain a variety of enzyme substrates for detection of ß-galactosidase (presence of coliforms) and ß-glucuronidase (presence of *E. coli*) (Manafi *et al.*, 1991; Manafi, 1996). The large scale screening carried out in this study has shown there is potential for such media to be developed for Bcc isolates. For example, an indoxylic chromogenic substrate linked to α-galactoside may be able to detect *B. stabilis*, or linked to ß-riboside to detect *B. stabilis*, *B.dolosa*, *B. ambifairia* and *B. anthina*, or to ß-xyloside to detect *B. cepacia, B. ambifaria* or *B. pyrrocinia*, although further investigation with such chromogenic substrates would be required. As Table 3.17 shows, there are differences between the Bcc species in their ability to hydrolyse the indoxylic substrates tested, however, the small numbers of organisms used in this initial study do not allow us to draw any conclusions, other than the correlation with the hydrolysis of the fluorogenic substrate equivalents was good. There may be potential for development of chromogenic media for organisms closely related to Bcc for which problems with identification exist, such as using an indoxylic derivative linked to *p*`-guanidinobenzoate for the identification of *R. pickettii*.

Non-indoxylic compounds are also used as substrates. Cyclohexenoesculin (CHE), for example, is a synthetic, compound, first described by James *et al.* (1996). The most widely used CHE-based substrate is perhaps CHE ß-glucoside, a synthetic alternative substrate to esculin. CHE ß-glucoside is hydrolysed by ß-glucosidase activity and the released aglycone forms a black chelate with iron (James *et al.*, 1997). Other

chromogenic substrates have the same mechanism of action as CHE substrates, i.e. they only produce colour on chelation with metal ions. For example, 1,2-dihydroxyanthraquinone (alizarin) substrates are hydrolysed to release the alizarin aglycone which forms purple chelates with iron (see Figure 3.16), and pink chelates with aluminium ions (see Figure 3.17) (James *et al.*, 2000).

The use of chromogenic substrates in diagnostic microbiology is currently limited and the number of chromogenic substrates with different metabolic moieties is much smaller than the corresponding number of fluorogenic substrates available. Chromogenic substrates are less sensitive than fluorogenic substrates, and thus need to be used at higher concentrations to visualise the reaction. However, non-diffusible chromogenic substrates can be used in agar-based media for the direct identification of species, resulting in brightly coloured colonies that can be directly visualised without a UV source. Different chromogenic substrates produce different coloured products, a combination of substrates can be used to target various enzyme activities, thereby improving media specificity and reducing the need for additional confirmatory tests. Furthermore, the number of new substrates is growing, greatly increasing the possible applications of enzyme substrates in culture media. In addition to the use of such substrates, the effectiveness of the media can be increased by the inclusion of conventional tests and antibacterial agents, so as to form a highly sensitive and selective medium for the detection of the target species. Figures 3.14-3.19 illustrate Bcc strains growing on isolation media incorporating various non-diffusible chromogenic substrates.

Figure 3.15: *B. cepacia* (ATCC 25416) colonies growing on media containing magenta caprylate (hydrolysed by caprylate esterase)

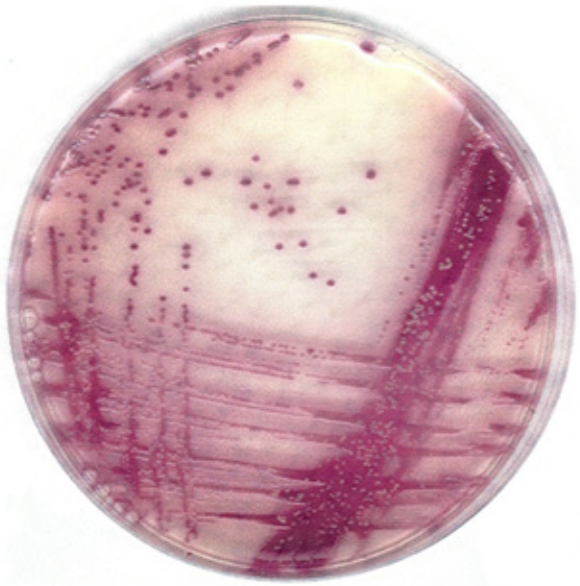


Figure 3.16: *B. cepacia* (ATCC 25416) colonies growing on media containing Alizarin glucoside (hydrolysed by ß-glucosidase) and ferric ammonium citrate

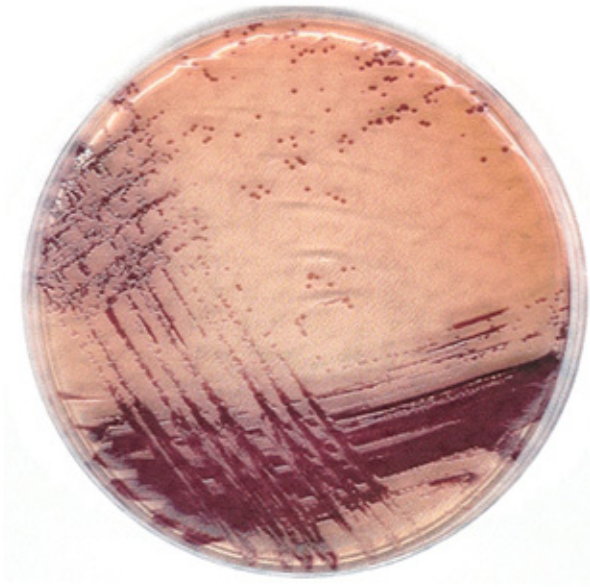


Figure 3.17: *B. cepacia* (ATCC 25416) colonies growing on media containing Alizarin glucoside (hydrolysed by ß-glucosidase) and potassium aluminium sulphate

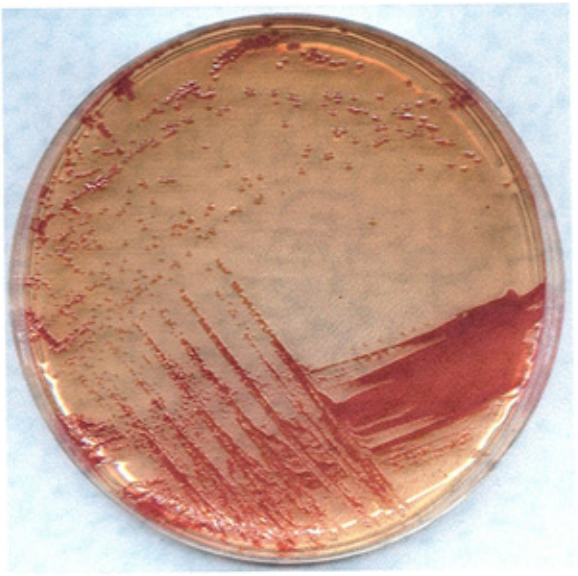


Figure 3.18: *B. cepacia* (ATCC 25416) colonies growing on media containing ß-alanyl-aminophenyl acridine (hydrolysed by alanine aminopeptidase)

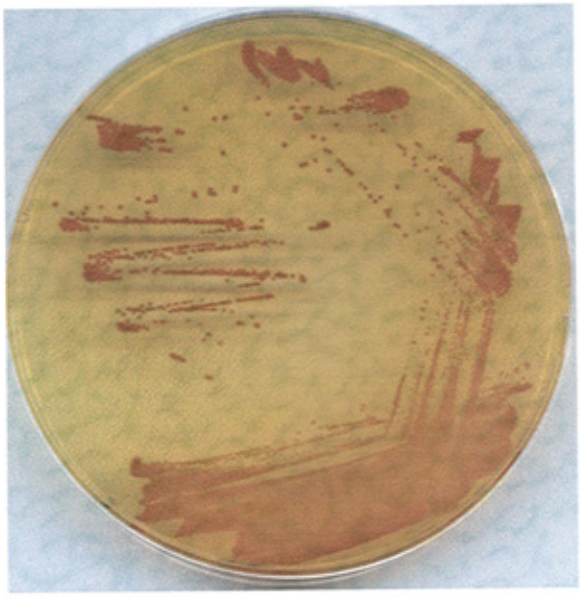


Figure 3.19: : *B. cepacia* (ATCC 25416) colonies growing on media containing Alanyl-CVO (hydrolysed by alanine aminopeptidase)

In conclusion, the results found with this extensive phenotypic screening exercise confirm the heterogeneity of the nine species comprising the Bcc as previously reported by a number of other working groups. The results also confirm the particularly close phenotypic relationship between *B. cepacia* and *B. cenocepacia*, (including both subtypes III-A and III-B).

Chromogenic substrate equivalents of those fluorogenic substrates which showed potential for discrimination between Bcc strains and/or closely related organisms in this study could be evaluated, including non-diffusable substrates for use within isolation media. In the future, indoxylic substrates such as those evaluated in this study, could be incorporated into isolation media for identification of Bcc strains, or even discrimination between the Bcc species. Differentiation of *P. aeruginosa* from Bcc is

not as significant as differentiation from other closely related organisms such as *Pandoraea* sp. and *R. pickettii*, as *P. aeruginosa* can be eliminated on isolation media using characteristics such as sensitivity to colistin or resistance to C390 for example. However, as *P. aeruginosa* is a significant pathogen in the lungs of CF patients, such tests may be of use for detection of this organism, for example in chromogenic isolation media. However, further investigation is warranted on these substrates, such as combining substrates within media, and also clinical trials using patient specimens, before such isolation media can be considered for routine application in the clinical laboratory setting.

CHAPTER 4

Antimicrobial synergy testing in Bcc and *P. aeruginosa* isolates with alafosfalin in combination with other cell wall-acting antibiotics

Introduction

In the majority of cases, following identification of a pathogenic isolate from a clinical specimen, antimicrobial susceptibility testing is performed by the clinical microbiology laboratory. Antibiotic resistance is a critical limiting factor in treating infections due to *B. cepacia* complex (Bcc) strains and *P. aeruginosa* infections in CF patients. Respiratory tract infection with eventual respiratory failure is the major cause of morbidity and mortality in CF. Infective exacerbations need to be treated promptly and effectively to minimize potentially accelerated attrition of lung function. The choice of antibiotic depends on *in vitro* sensitivity patterns. However, physicians treating patients with CF are increasingly faced with infection with multidrug-resistant isolates of *P. aeruginosa.* In addition innately resistant organisms such as the Bcc are becoming more prevalent (Conway *et al.*, 2003). This is probably resulting from greater patient longevity and increased antibiotic use for acute exacerbations and maintenance care. The multiple antibiotic resistance of Bcc isolates has been attributed to an impermeable outer membrane, cellular target alteration, an efflux pump mechanism and/or inducible chromosomal β-lactamase (Yu *et al.*, 1999). In *P. aeruginosa* isolates, resistance is mainly a result of production of β-lactamase, the mutation of porin proteins, and/or production of alginate biofilms (Hoiby, 2002).

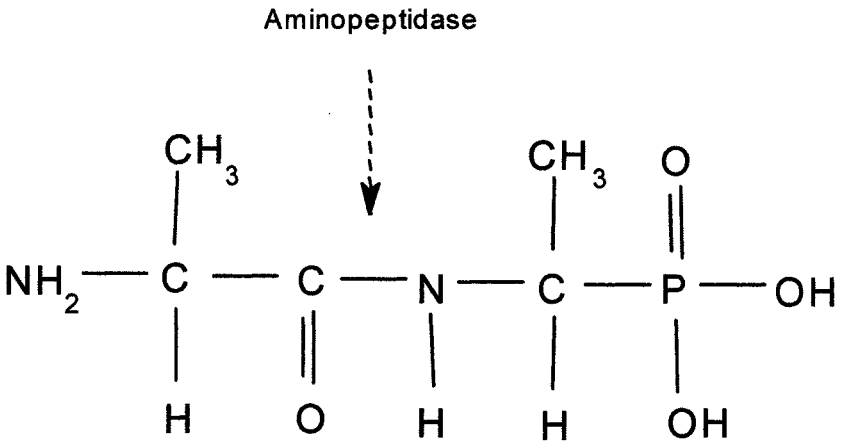
CF infections caused by *P. aeruginosa* and Bcc strains, are most successfully treated using two or three drug combinations. Fortunately, a variety of antipseudomonal agents are available. For acute infectious exacerbations, the usual first line therapy is an aminoglycoside combined with ceftazidime or an antipseudomonal penicillin; for chronic infection, ciprofloxacin may be more suitable in the first instance (Bosso *et al.*,

1987; Hoiby, 2002). The most useful extended-use penicillins in the treatment of pseudomonal infections are the piperazine penicillin piperacillin, and the carboxypenicillin ticarcillin (Hoiby, 2002). Bcc strains that have not been challenged with antibiotics are typically susceptible only to piperacillin, piperacillin-tazobactam, cefoperazone, ceftazidime, chloramphenicol, and trimethoprim-sulfamethoxazole, with variable susceptibilities to imipenem and meropenem (Miller and Gilligan, 2003). Ceftazidime in particular has good *in vitro* activity and is resistant to most ß-lactamases. It is easily administered, does not require monitoring of serum drug concentrations and has low toxicity (Banerjee and Stableforth, 2000). Previous studies have shown the value of antibiotic combinations as opposed to single drug therapy, these include ß-lactam-ciprofloxacin, ß-lactam-ciprofloxacin-rifampicin, ß-lactam-aminoglycoside, ß-lactam-ciprofloxacin-tobramycin, meropenem-ciprofloxacin, and aztreonam-tobramycin against Bcc strains (Aronoff and Klinger, 1984; Kumar *et al.*, 1989; Bosso *et al.*, 1990; Lu *et al.*, 1997; Bonacorsi *et al.*, 1999; Banjeree and Stableforth, 2000) and ß-lactam-aminoglycoside-rifampicin, meropenem-ciprofloxacin, and aztreonam-tobramycin against *P. aeruginosa* (Yu *et al.*, 1984; Zuravleff *et al.*, 1984; Lang *et al.*, 2000). Multiple combination bactericidal antibiotic testing (MCBT) has shown meropenem-minocycline, meropenem-amikacin and meropenem-ceftazidime combinations to be bactericidal against high proportions of Bcc strains, and that triple-antibiotic combinations, in particular tobramycin-meropenem-ceftazidime, are more effective than double and single antibiotic combinations against Bcc *in vitro* (Aaron *et al.*, 2000). MCBT demonstrated tobramycin plus either meropenem, piperacillin/tazobactam, or ciprofloxacin, were the most effective double antibiotic combinations against *P. aeruginosa*, and that adding a third antibiotic did not significantly improve inhibition *in vitro*.

Mode of action of alafosfalin

Antibiotics of the group that includes the ß-lactams, monobactams, D-cycloserine and vancomycin, act by interfering with the biosynthesis of bacterial cell walls. This is achieved by inhibiting the formation, or the subsequent utilization in cross linking, of the terminal D-alanyl-D-alanine units which occur in the peptide chains of the cell-wall peptidoglycans of bacteria (Strominger, 1962; Ghuysen *et al.*, 1967). Alafosfalin is representative of a novel series of antibacterial phosphonopeptides that was designed to mimic the terminal dipeptide moiety (D-alanyl-D-alanine) of bacterial cell wall peptidoglycan (Allen *et al.*, 1978). The mechanism of action of alafosfalin may be regarded as involving at least three stages (i) active transport by peptide permeases, (ii) intracellular peptidase cleavage (see Figure 4.1), and (iii) action of L-1-aminoethylphosphonate on alanine racemase (Atherton *et al.*, 1979a)

Figure 4.1: Chemical structure of L-alanyl-1-aminoethylphosphonic acid (alafosfalin) and the bacterial aminopeptidase site of action



The results found by Atherton *et al.* (1979a) established that alafosfalin selectively inhibits peptidoglycan biosynthesis in both Gram negative and Gram positive bacteria. In this study alafosfalin was shown to be actively transported into cells by stereospecific peptide permeases and subsequently hydrolysed by intracellular aminopeptidases to yield L-1-aminoethylphosphonic acid. This alanine mimetic rapidly accumulates inside susceptible cells to yield a concentration 100- to 1000-fold in excess of that of the precursor peptide in the surrounding medium. In the case of susceptible Gram negative organisms, 1-aminoethylphosphonic acid is incorporated into uridine diphosphate-N-acetylmuramyl-aminoethylphosphonate. The primary intracellular target site of 1-aminoethylphosphonic acid is alanine racemase. This was reversibly and competitively inhibited in *E. coli* and *P. aeruginosa* and irreversibly inhibited in a time-dependent manner in *S. aureus* and *E. faecalis* Atherton *et al.* (1979a).

Antimicrobial interactions with Alafosfalin

Double blockade of D-alanine utilization by combinations of alafosfalin and D-cycloserine and ß-lactams has been shown to produce synergistic effects (Ghuysen and Shockman 1973; Allen *et al.*, 1979a), and other inhibitors of racemase are known to potentiate penicillin (Soper and Manning, 1976). Several workers have studied the activity of alafosfalin in combination with ß-lactams against a variety of Gram positive and Gram negative organisms, including *Proteus* sp., *E. coli, Serratia* sp., *Klebsiella* sp., *Enterobacter* sp., *Acinetobacter* sp., *S. aureus, E. faecalis*, and *P. aeruginosa* (Allen *et al.*, 1979a; Maruyama *et al.*, 1979; Arisawa *et al.* 1982). These studies concluded synergism between alafosfalin and ß-lactams is evident with a wide range of genera and species. It has also been shown that although alafosfalin is inactive against *P.*

aeruginosa, synergy is often observed with such combinations. For example, Arisawa *et al.* (1982) found that although alafosfalin is not active against *P. aeruginosa* on its own, potentiation was seen when combined with cefsulodin.

Although there are a limited number of previous studies published on the activity of alafosfalin on *P. aeruginosa* and in particular Bcc strains, it has been observed in a number of studies that other Gram negative bacilli which are resistant to alafosfalin alone, are susceptible to the antibiotic when in combination with ß-lactams. For example, Atherton *et al.* (1981) studied the synergistic activity of alafosfalin with cephalexin, mecillinam and ampicillin and found that *Proteus mirabilis*, which is resistant to alafosfalin *in vitro*, was inhibited by alafosfalin in combination with the three ß-lactam antibiotics. This property of alafosfalin, i.e. potentiation of the activity of other cell wall active agents such as ß-lactams against many bacteria, could make it a viable option for the treatment of lung infections in cystic fibrosis patients.

Pharmacokinetics of alafosfalin

Allen *et al.* (1979b) studied the metabolic fate and pharmacokinetics of alafosfalin in various species including humans. The compound was found to be rapidly absorbed from the injection site after subcutaneous and intramuscular administration and gave peak plasma concentrations at 15 to 30 minutes after dosing. Distribution studies showed that the drug did not bind to plasma albumin significantly and hence there was a high drug concentration found in most tissues, including lung tissue, and inflammatory exudates except in the central nervous system and liver. Administration of a 200 mg intramuscular or 500 mg oral dose produces a concentration of intact phosphonopeptide

in human plasma and urine, which is in excess of the *in vitro* minimum inhibitory concentrations (MICs) of many pathogenic organisms. Studies in human volunteers have shown that alafosfalin is well absorbed from the gastrointestinal tract, but some metabolite hydrolysis occurs before the drug reaches the general circulation.

Alafosfalin was well absorbed after oral administration but was extensively hydrolysed to alanine and L-1-aminoethylphosphonic acid before it reached the general circulation. Oral bioavailability is approximately 50 % and largely independent of dose (Allen *et al.*, 1979b). Distribution studies showed that alafosfalin is mainly excreted by the kidney. Alafosfalin has an elimination half life of about 60 minutes and does not accumulate during chronic administration. Unchanged drug and metabolite are mainly excreted in the urine (Allen *et al.*, 1980). Amongst the phosphonopeptides, alafosfalin is stable to intestinal and renal peptidases (Allen *et al.*, 1980). The rate of absorption and elimination of alafosfalin in humans were also very similar to published data on ß-lactam antibiotics. This suggests that pharmacokinetics can be matched to provide synergistic combinations for clinical use.

The chequerboard technique

The term synergy has several different interpretations and definitions, which are influenced by the methods used (Owens *et al.* 1997). When setting up any experimental system to study antibiotic-antibiotic interactions, the choice of antibiotic concentrations is very important, whether single or multiple concentrations will be used, if these will be based on measures of drug potency or pharmacokinetic concentrations, and the appropriate end points (MacGowan, 2003). Some methods are best adapted for testing

single isolates while others allow a number of strains to be tested. Whichever method is selected it should be of known reproducibility and should hopefully have known individual clinical correlations or be of value in population-based prediction (MacGowan, 2003). It should be clear as to the definition of synergy; addition, indifference or antagonisms in the test system employed (Hall *et al.*, 1983).

Classically, two methods of studying antibacterial-antibacterial interactions are used, that is the chequerboard method and the bacterial time-kill curve, both having been used previously to investigate interactions in Bcc isolates (Bonacorsi *et al.*, 1999; Mackay *et al.*, 2000) and *P. aeruginosa* (Saiman *et al.*, 1996). Synergy, as defined by the chequerboard methodology, represents at least a fourfold reduction in MIC when agents are combined compared with activities of either agent alone. The chequerboard can be in the form of microdilution (Gould *et al.*, 1991) or agar dilution (Wooton *et al.*, 1995). Chequerboard tests often employ MIC multiples as the drug concentrations, hence allowing a range of concentrations to be used (MacGowan, 2003). The chequerboard technique is a useful means of assessing antibiotic interactions, especially when large numbers of bacterial strains or antimicrobial combinations are to be tested. Combinations of antimicrobial agents may be investigated in patients receiving multiple antibiotics to enable the treatment of all suspected pathogens, to prevent the emergence of resistance, or to help improve clinical efficacy. The advantage of using a chequerboard technique for studying antibiotic interactions is that it is a commonly used method so data produced can be easily compared with previously published studies (MacGowan, 2003) .Although time consuming to perform and therefore not ideally suited to the routine laboratory, the technique is easy to understand and simple to perform. It has found extensive use in the study of antibiotic combinations for treatment

of multi-resistant organisms, including *P. aeruginosa* and Bcc strains (Owens *et al.*, 1997; Di Pentima *et al.*, 1998; Mackay *et al.*, 2000).

Interpretation of chequerboards

Chequerboards are interpreted by calculation of the fractional inhibitory concentration (FIC) index, as first described by Elion *et al.* (1953). The FIC is the sum of the MIC of each drug in combination divided by the MIC of the drugs acting alone (see Figure 4.2). Once the FIC index is calculated, the criteria to define synergy and antagonism can be stated strictly as FIC < 1 indicates synergy, FIC >1 indicates antagonism and FIC = 1 indicates indifference (Berenbaum *et al.*, 1978; 1983). Alternatively a one dilution or greater error can be built in to the analysis, and so synergy is indicated by an FIC index < 0.5, addition, by 0.5 - 1.0, indifference by 1.0 - 2.0, and antagonism by an FIC index of > 2.0 (Krogstat and Moellering, 1986). This interpretation is used by the CF Referral Centre for Susceptibility and Synergy Studies of Resistant Organisms (Saiman, 1998). Mackay *et al.* (2000) demonstrated that chequerboard techniques showed good reproducibility and good correlation between FIC indices.

Figure 4.2: Formula used for calculating Fractional Inhibitory Concentration (FIC)

$$FIC = \frac{\text{MIC of agent "A" in combination}}{\text{MIC of agent "A" alone}} + \frac{\text{MIC of agent "B" in combination}}{\text{MIC of agent "B" alone}}$$

(Taken from *CF Referral Centre for Susceptibility and Synergy Studies of Resistant Organisms Guidelines*, Columbia University, 1998)

Experimental objective

To evaluate alafosfalin in combination with a number of cell wall-acting antibiotics, using the agar dilution chequerboard technique to investigate potential treatment alternatives for CF patients infected with *P. aeruginosa* and/or Bcc strains. This was achieved using 19 clinical isolates of *P. aeruginosa* cultured from cystic fibrosis patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit, Newcastle upon Tyne, and 19 genetically defined Bcc strains.

Materials

Bacterial strains

Nineteen *P. aeruginosa* isolates were cultured from pre-operative broncho alveolar lavages (BAL) of CF lung transplant patients from the Freeman Hospital, Newcastle upon Tyne, (see Appendix 3.1, strains 1–19) and were identified using Analytical Profile Index (API) 20NE strips and associated software (bioMérieux, La Balme-les-Grottes, France) and C390 sensitivity tests (Biosynth AG, UK), and 19 Bcc strains which were taken from the published experimental panel of 30 Bcc isolates from the BCCM (Mahenthiralingam *et al.*, 2000) (see Appendix 4.1 for strains selected from this panel). *P. aeruginosa* NCTC 10662 was included in the collection as a reference strain. Each strain was stored on lenticules as previously described (Codd *et al.*, 1998; Lightfoot *et al.*, 2001) and stored at – 20 °C.

Growth media

Columbia agar was obtained from Oxoid Ltd (Basingstoke, UK). Defibriniated horse blood was obtained from TCS Biosciences Ltd (Buckingham, UK). An "antagonist-free" agar was used as described by Atherton *et al* (1980). Table 4.1 shows the constituents of this agar base.

Table 4.1: Formula of agar base for synergy testing with alafosfalin (per litre of water)

Ingredient	Amount (mg)
L-Arginine	100
L-Aspartic acid	100
L-Cysteine	100
Glycine	100
L-Histidine	100
L-Isoleucine	100
L-Leucine	100
L-Lysine-HCL	100
L-Methionine	100
L-Phenylalanine	100
L-Proline	100
L-Serine	100
L-Threonine	100
L-Tryptophan	100
L-Tyrosine	100
L-Valine	100
Guanine	10
Uracil	10
Cytosine	10
Adenine	10
Sodium Citrate	500
Magnesium sulphate	100
Ammonium sulphate	1000
Yeast Extract	100
Dipotassium hydrogen phosphate	7000
Potassium dihydrogen phosphate	2000
Glucose	5000
Bacteriological agar	10000

All amino acids were obtained from Sigma-Aldrich Company Ltd. (Poole, UK) Sodium citrate, magnesium sulphate, ammonium sulphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate and glucose were obtained from BDH (Poole, UK), yeast extract and bacteriological agar were obtained from Oxoid (Basingstoke, UK).

Antimicrobials

Alafosfalin was obtained from Sigma-Aldrich Company Ltd. (Poole, UK), aztreonam was obtained from Squibb (Hownslow, UK), meropenem was obtained from Astra Zeneca (Luton, UK), ciprofloxacin was obtained from Bayer (Newbury, UK), ticarcillin/clavulanic acid (Timentin) was obtained from SmithKline Beecham (Hertfordshire, UK), ceftazidime was obtained from (Eli Lilly & Co. Ltd. (Basingstoke, UK), cefsulodin was obtained from Takeda, (Woodburn Green, UK), tobramycin was obtained from Cox Pharmaceuticals (Exeter, UK) and piperacillin/tazobactam (Tazocin) was obtained from Lederle (Maidenhead, UK). The antimicrobials were reconstituted according to manufacturers instructions.

Equipment

All substrates were prepared using a Satorius 2434 electronic balance; accurate to 0.1 mg (Satorius Ltd, Epsom, UK). Small volumes were dispensed using calibrated Gilson semi-automatic pipettes (P20, P200 and P1000) with sterile disposable tips (Gilson Medical Electronics, Villiers-le-Bel, France). Large volumes were dispensed using sterile disposable 10 ml pipettes (L.I.P. Ltd, Shipley, UK). Plastic consumables, including 25 ml universals, 5 ml bijoux and sterile petri dishes, were obtained from Bibby Sterilin Ltd., Aberbargoed, UK. All organisms were prepared to a specific suspension density using a Densimat (bioMérieux, Marcy L`Etoile, France). All plates were incubated in a LEEC 30 ^{O}C incubator (laboratory and Electrical Engineering Company, Nottingham, UK). Organisms were inoculated onto the "antagonist-free" agar containing using a multipoint inoculator (Denley-Tech Ltd, UK).

Methods

Bacterial strain preparation

All strains were cultured as described in previous chapters.

Serial dilutions of antibiotics

Serial dilutions were prepared from freshly prepared stock solutions at 20 x the desired highest final concentration, in sterile distilled water. The extreme concentrations of the final antimicrobial ranges were 2 and 32 mg/l for alafosfalin, 1 and 16 mg/l for ceftazidime, 0.25 and 4 mg/l for ciprofloxacin, 1 and 16 mg/l for aztreonam, 2 and 32 mg/l for cefsulodin, 4 and 64 mg/l for ticarcillin/clavulanic acid, 0.5 and 8 mg/l for meropenem, 1 and 16 mg/l for piperacillin/tazobactam, and 0.25 and 4 mg/l for tobramycin. These were prepared as follows:

Alafosfalin (640 mg/l) - 20 mg into 20 ml sterile distilled water (1000 mg/l)

- 16 ml of this into 9 ml sterile distilled water (640 mg/l)

Ceftazidime (320 mg/l) – 10 ml sterile distilled water added to 1 g in vial (100 g/l)

- 200 µl of this into 19.8 ml of sterile distilled water (1000 mg/l)

- 8 ml of this into 17 ml of sterile distilled water (320 mg/l)

Ciprofloxacin (80 mg/l) - supplied as 2000 mg/l solution

- add 1 ml of this to 24 ml sterile distilled water (80 mg/l)

Aztreonam (320 mg/l) - 5 ml sterile distilled water added to 1 g (200 g/l)

- 200 µl of this into 19.8 ml sterile distilled water (2000 mg/l)

- 4 ml of this into 21 ml sterile distilled water (320 mg/l)

Cefsulodin (640 mg/l) - 20 mg into 20 ml sterile distilled water (1000 mg/l)

- 16 ml of this into 9 ml of sterile distilled water (640 mg/l)

Ticarcillin/clavulanic acid (1280 mg/l) - 10 ml of sterile distilled water added to 3.2 g (320 g/l)

- 100 µl of this into 24.9 ml sterile distilled water (1280 mg/l)

Merepenem (160 mg/l) - 10 ml sterile distilled water added to 1 g (100000 mg/l)

- 40 µl of this into 24.6 µl sterile distilled water (160 mg/l)

Piperacillin/tazobactam (320 mg/l) – 20 ml sterile distilled water added to 2.25 g (112500 mg/l)

- 70 µl of this into 24.3 ml (320 mg/l)

Tobramycin (80 mg/l) – 180 mg into 2 ml sterile distilled water (40000 mg/l)

- 50 µl of this into 24.95 ml (80 mg/l)

Preparation of chequerboard

A standard "half-chequerboard" technique was used to test for synergy. A 1 ml aliquot of each preparation was added to 18 ml of the antagonist-free agar to produce unique combinations of the paired antibiotics. Alafosfalin was tested with each antibiotic. In addition, ceftazidime was tested in combination with tobramycin, with and without the presence of alafosfalin. Each antibiotic was also tested alone by adding 1ml of each concentration of antibiotic to 1 ml of sterile, distilled water plus 18 ml of agar. MICs of alafosfalin for Bcc and *P. aeruginosa* strains were determined. An antibiotic-free

control plate was also included. Figure 4.3 illustrates the distribution of antibiotics within the chequerboard. These antibiotic/agar combinations were poured into individual Petri dishes. Once the medium had set, the plates were dried to remove surface moisture, and then inoculated immediately as described below.

Figure 4.3. Chequerboard titration antibiotic concentrations for alafosfalin in combination with meropenem

Alafosfalin (mg/l) \ Meropenem (mg/l)	0	0.5	1	2	4	8
0		0.5	1	2	4	8
2	0.5, 2	1, 2	2, 2	4, 2	8, 2	
4	0.5, 4	1, 4	2, 4	4, 4	8, 4	
8	0.5, 8	1, 8	2, 8	4, 8	8, 8	
16	0.5, 16	1, 16	2, 16	4, 16	8, 16	
32	0.5, 32	1, 32	2, 32	4, 32	8, 32	

Inoculum and incubation

A single colony from each bacterial strain was suspended in an aliquot of sterile water and corrected to a density equivalent to McFarland standard 0.5 (approximately 1.5 x 10^8 cfu/ml). A multipoint inoculator was used to inoculate the antagonist free agar with 1 µl of each organism suspension (giving a final inoculum of approximately 1.5 x 10^5 cfu). One set of plates was multipoint inoculated with 19 Bcc strains, the other with 19 *P. aeruginosa* isolates. Both sets of plates were inoculated also with the *P. aeruginosa* NCTC 10662 Type strain. These plates were incubated at 37 °C for 18 hours.

MIC and FIC determination

After incubation the MIC of the antibiotics for the Bcc strains and P. aeruginosa isolates were read, both alone and in combination. The MIC was the lowest concentration at which less than 5 colonies could be seen macroscopically on the point of inoculation. From these results, FICs were calculated and the antibiotic interaction present was determined using these values. All combinations were performed in duplicate to ensure reproducibility. All evaluations of antimicrobial combinations were performed in duplicate.

MIC of alafosfalin

The MIC of alafosfalin against all Bcc and *P. aeruginosa* isolates used in this study was investigated by making up the antagonist-free agar and incorporating serial dilutions of alafosfalin from 32 mg/l up to 2048 mg/l. Ten times the top concentration of alafosfalin (20.48 g/l) was prepared by adding 204.8 mg into 10 ml sterile distilled water. The desired concentration (2048 mg/l) was then achieved by adding 2 ml of each preparation to 18 ml of antagonist-free agar. These antibiotic/agar combinations were poured into individual Petri dishes, the plates were set, dried and were then inoculated and incubated as described earlier under "Inoculum and incubation".

Results

For each antibiotic combination evaluated, growth of the isolates was recorded as plus or minus, depending upon whether more than five colonies were visible on the point of inoculum. Figure 4.4 illustrates an example of this using the results for the combination of alafosfalin with cefsulodin for Bcc strain no. 16.

Figure 4.4: Growth recordings for cefsulodin with alafosfalin for Bcc strain no. 16.

	Alafosfalin conc (mg/l)					
Cefsulodin conc (mg/l)	**0**	**2**	**4**	**8**	**16**	**32**
0	+	+	+	+	+	-
2	+	+	+	+	+	-
4	+	+	+	+	+	-
8	+	+	+	+	+	-
16	+	+	+	+	-	-
32	+	+	+	+	-	-

From these results, the FIC indices were evaluated for all combinations of antibiotics, against all strains tested (see Appendix 4.2). The FIC for this strain, using the formula described, is 0.75. Although an FIC of 0.5 - < 1 usually denotes addition with two antibiotics, since both *P. aeruginosa* and Bcc strains are resistant to alafosfalin (MICs > 2048 mg/l) it can be assumed that if the MIC of an antibiotic is lowered, even though this may give an FIC index of 0.5 - < 1, synergy must be occurring. This has therefore been referred to as "minor synergy" throughout this project. The FIC of 0.75 in this

example therefore denotes that the antibiotic interaction occurring against Bcc strain 16, between alafosfalin and cefsulodin, is minor synergy.

Appendix 4.2 shows the FIC values for all isolates with all antibiotic combinations. These results show that the majority of antibiotic interactions seen in this study were that of indifference (FIC = 1.0-2.0). Any synergy observed (including minor synergy) has been summarized in Table 4.2. The run with the highest level of synergy for each combination has been calculated as a percentage in Table 4.3. There was no antagonism observed with any of the antibiotic combinations evaluated.

Table 4.2: Number of strains showing synergy with alafosfalin/antibiotic combinations

Antimicrobial combination	Run 1		Run 2	
	Bcc	*P.aeruginosa*	Bcc	*P.aeruginosa*
Aztreonam/Alafosfalin	3(2)	7(2)	2(0)	4(1)
Cefsulodin/Alafosfalin	2(2)	2(1)	2(2)	0(0)
Ceftazidime/Alafosfalin	7(4)	1(0)	8(5)	2(2)
Ciprofloxacin/Alafosfalin	0(0)	0(0)	0(0)	0(0)
Meropenem/Alafosfalin	6(3)	5(1)	6(3)	2(0)
Tazocin/Alafosfalin	1(1)	2(2)	2(2)	3(0)
Timentin/Alafosfalin	2(2)	3(0)	2(2)	4(1)
Tobramycin/Alafosfalin	0(0)	3(0)	0(0)	2(0)
Tobramycin/Ceftazidime	7(0)	7(1)	6(0)	8(1)
Tobramycin/Ceftazidime/Alafosfalin	2(0)	10(5)	3(0)	11(5)

(#) = strains with FIC < 0.5

Table 4.3: Highest numbers of isolates from the two test runs on which the antibiotics exerted a synergistic effect, expressed as a percentage

Antimicrobial combination	Organism	
	Bcc	*P.aeruginosa*
Aztreonam/Alafosfalin	16 (11)	37 (11)
Cefsulodin/Alafosfalin	11 (11)	11 (5)
Ceftazidime/Alafosfalin	42 (26)	11 (11)
Ciprofloxacin/Alafosfalin	0 (0)	0 (0)
Meropenem/Alafosfalin	32 (16)	26 (5)
Tazocin/Alafosfalin	11 (11)	16 (0)
Timentin/Alafosfalin	11 (11)	21 (5)
Tobramycin/Alafosfalin	0 (0)	16 (0)
Tobramycin/Ceftazidime	37 (0)	42 (5)
Tobramycin/Ceftazidime/Alafosfalin	16 (0)	58(32)

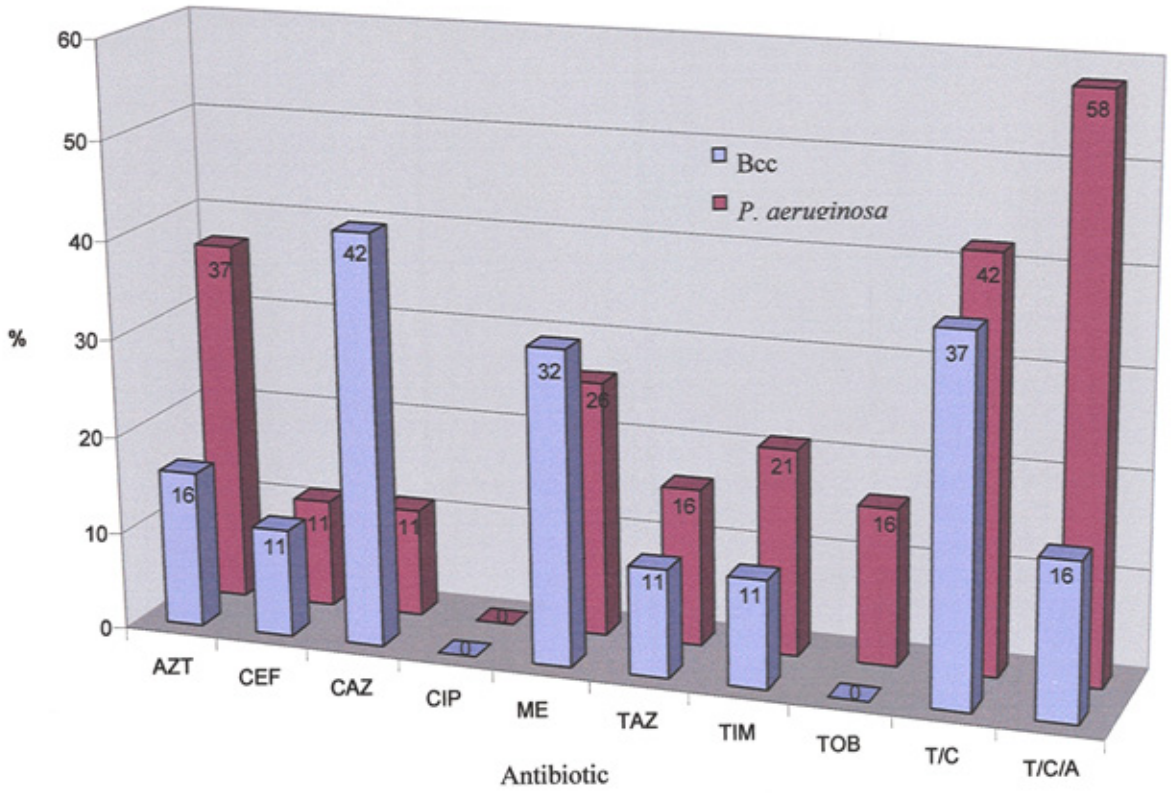
(#) = strains with FIC < 0.5

Figures 4.4 and 4.5 illustrate the percentage of Bcc strains and *P. aeruginosa* isolates on which the antibiotic combinations exerted a synergistic effect, with Figure 4.5 displaying those combinations which gave FIC index < 0.5.

For the 19 Bcc strains, the combination of alafosfalin with ceftazidime was the most effective antibiotic double combination, having demonstrated a synergistic effect in 42 % of strains. This combination had a FIC index < 0.5 in 26 % of strains. Meropenem with alafosfalin was also shown to be a useful combination, with synergy occurring in 32 % of strains. Meropenem with alafosfalin had a FIC index < 0.5 in 16% of strains. Aztreonam, cefsulodin, tazocin, and timentin showed some synergy when in combination alafosfalin, but only against small numbers of strains. Ciprofloxacin and tobramycin were shown to be of little use when in combination with alafosfalin. It is interesting to note that tobramycin when combined with ceftazidime, without the presence of alafosfalin showed synergy in a high proportion of Bcc strains, i.e. against 37 % of strains,

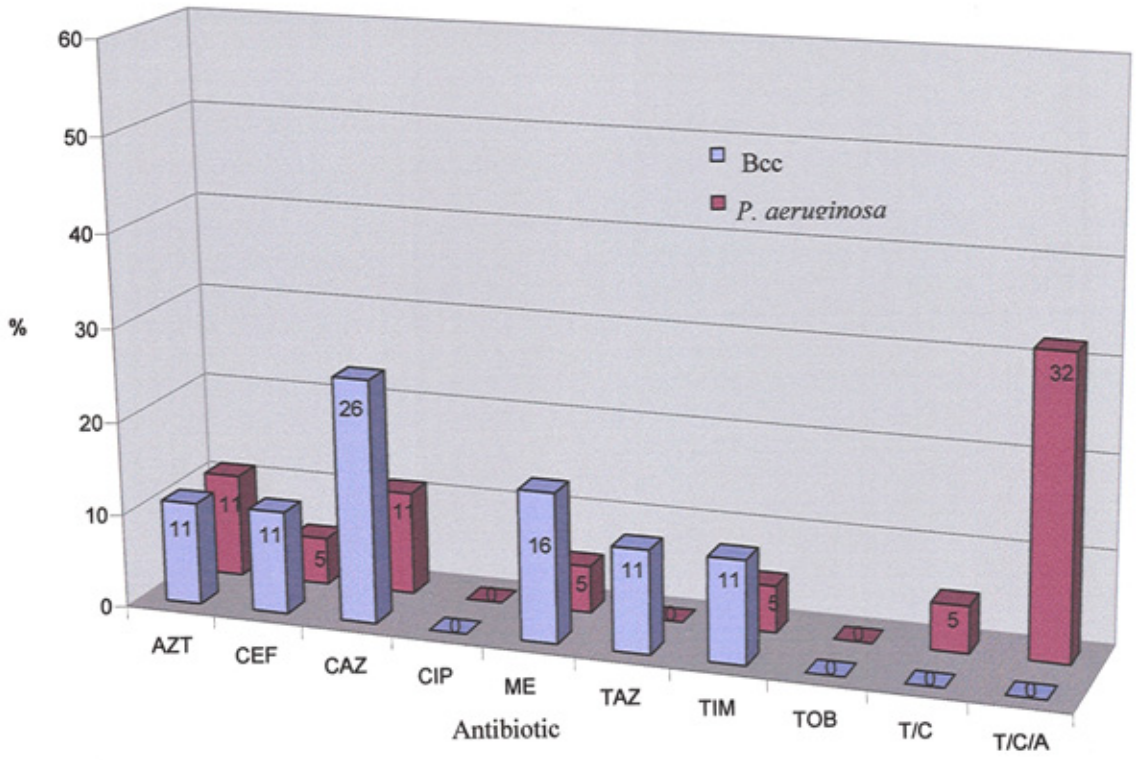
although this was all minor synergy (FIC > 0.5 < 1), but with the addition of 32 mg/l of alafosfalin, the number of strains demonstrating synergy was reduced to 16 %.

Figure 4.5a: Percentage of Bcc and *P. aeruginosa* strains in which synergy was observed when tested *in vitro* with alafosfalin/antibiotic combinations



Key:
CAZ, Ceftazidime; ME, meropenem; TAZ, piperacillin/tazobactam; TIM, Ticarcillin/clavulanic acid; CIP, ciprofloxacin; CEF, cefsulodin; AZT, aztreonam; TOB, tobramycin; T/C, tobramycin with ceftazidime; T/C/A, tobramycin, ceftazidime and alafosfalin

Figure 4.5b: Percentage of Bcc and *P. aeruginosa* strains with alafosfalin/antibiotic combination FICs < 0.5 when tested *in vitro* with alafosfalin/antibiotic combinations



Key:
CAZ, Ceftazidime; ME, meropenem; TAZ, piperacillin/tazobactam; TIM, Ticarcillin/clavulanic acid; CIP, ciprofloxacin; CEF, cefsulodin; AZT, aztreonam; TOB, tobramycin; T/C, tobramycin with ceftazidime; T/C/A, tobramycin with ceftazidime and alafosfalin

For the 19 *P. aeruginosa* isolates, the combination demonstrating synergy against the highest percentage of strains was alafosfalin with tobramycin and ceftazidime (58 %). A synergistic effect was observed in a higher percentage of *P. aeruginosa* strains with this triple antibiotic combination than with the double combination of tobramycin and

ceftazidime, without the addition of 32 mg/l alafosfalin (42 %). This is in contrast to the synergy observed using these antibiotic combinations against Bcc strains, as synergy was demonstrated in a higher percentage of organisms using ceftazidime and tobramycin as a double combination (37 %) compared with that observed when alafosfalin was also incorporated (16 %). This antibiotic combination with alafosfalin in *P. aeruginosa* isolates also demonstrated a much higher percentage of FIC values < 0.5, i.e. 32 %, compared to 5 % in Bcc strains. Aztreonam proved useful when in combination with alafosfalin, demonstrating synergy in 37 % of *P. aeruginosa* isolates, and also meropenem with alafosfalin, with synergy occurring in 26 % of isolates. Piperacillin/tazobactam and ticarcillin/clavulanic acid, each in combination with alafosfalin, demonstrated synergy in lower percentages of *P. aeruginosa* isolates, 16 % and 21 % respectively. Cefsulodin, ceftazidime and tobramycin each showed a synergistic effect when in combination with alafosfalin, but only against a small number of isolates. Ciprofloxacin was of little use when combined with alafosfalin against *P. aeruginosa* isolates, as observed for Bcc strains.

Figures 4.6 to 4.15 illustrate the effect of alafosfalin on the MICs of the antibiotics in combination, in relation to the breakpoints of these antibiotics for a selected number of strains which showed the most marked effect.

Figure 4.6: Effect of alafosfalin concentration on the M.I.C. of ceftazidime for Bcc strains

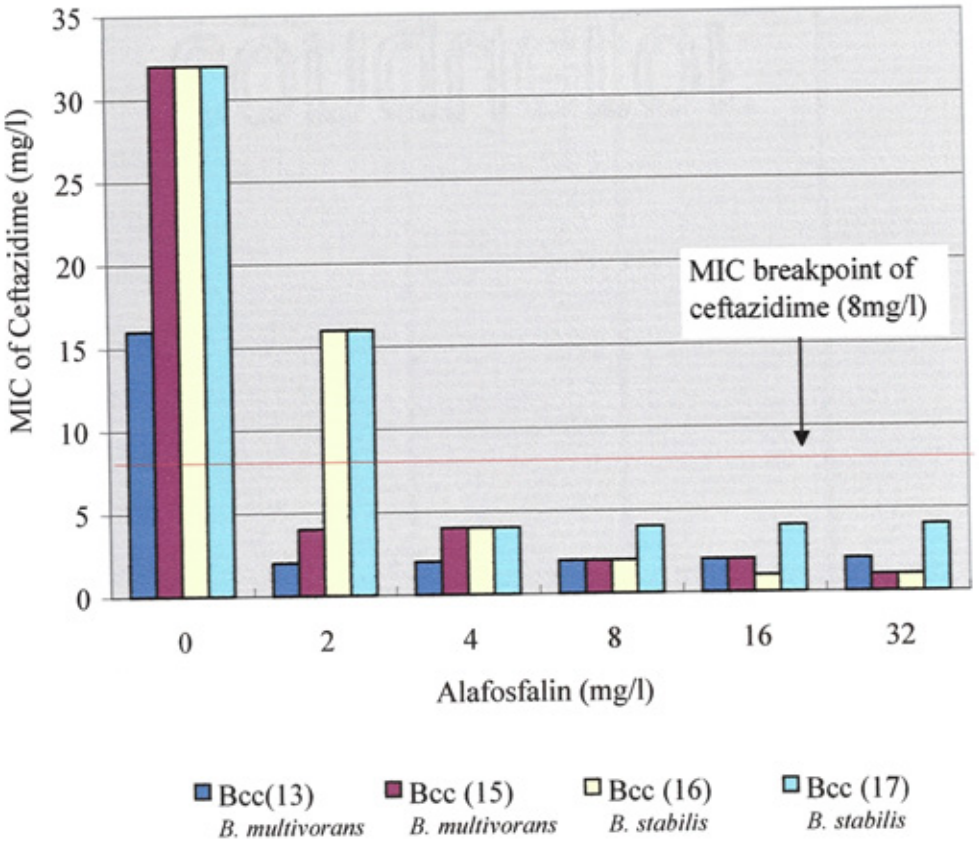
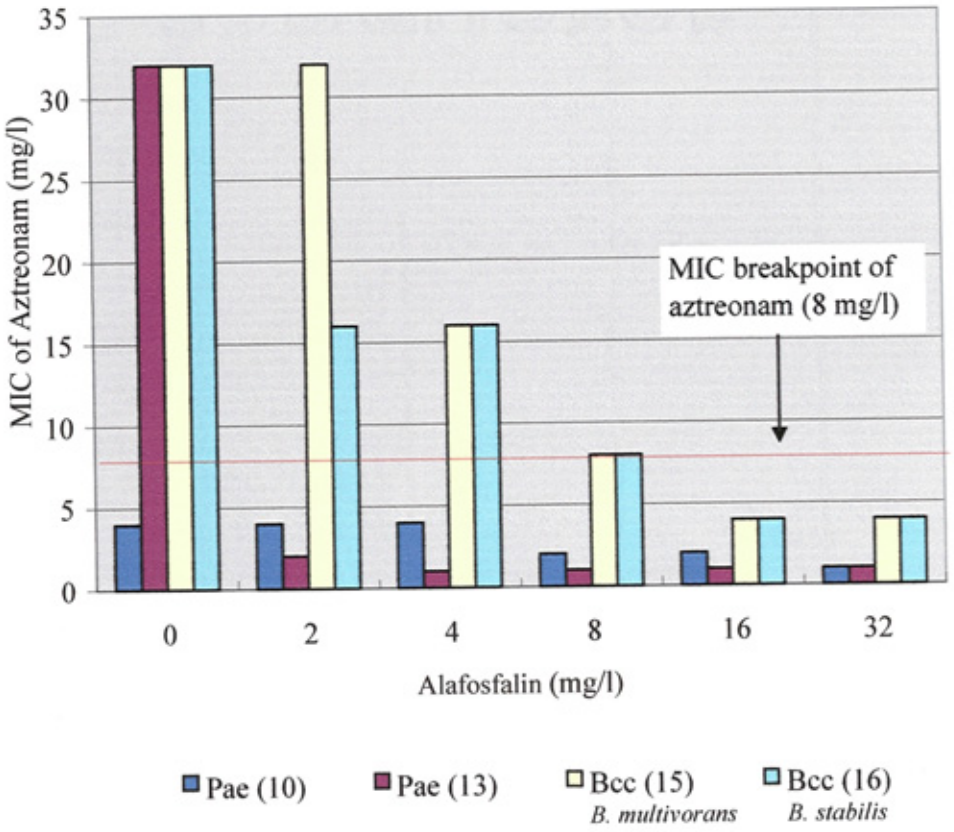
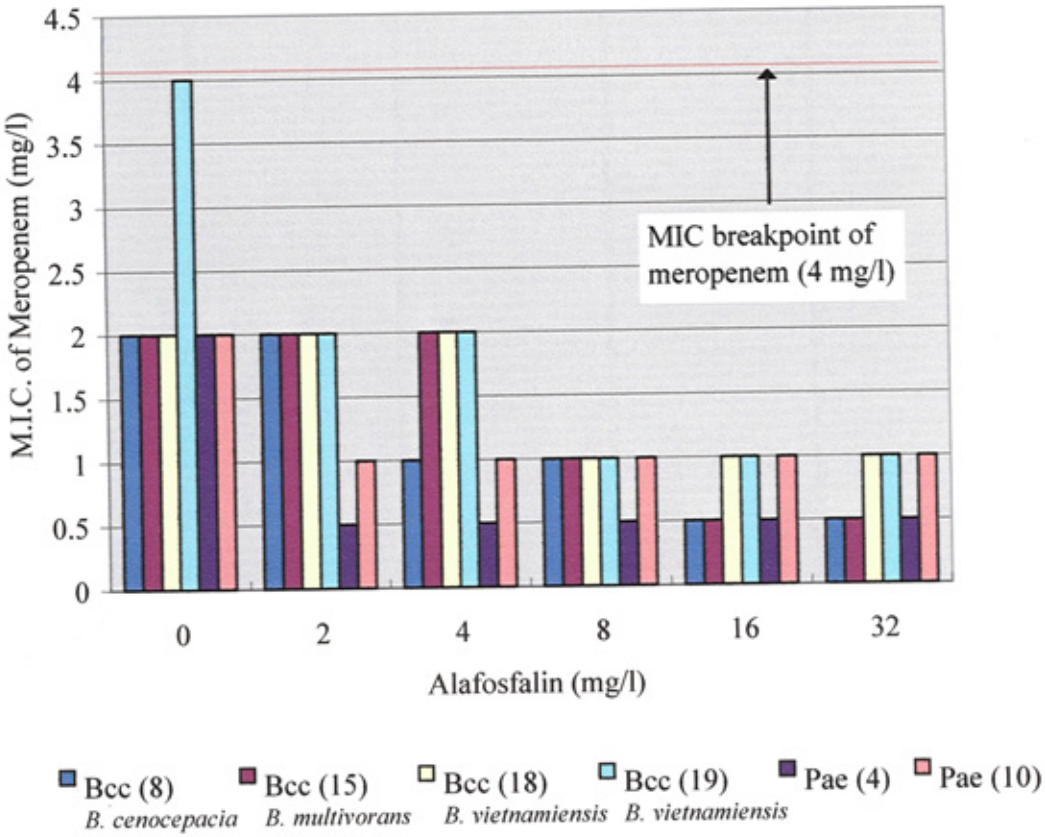


Figure 4.7: Effect of alafosfalin concentration on the M.I.C. of aztreonam for Bcc and *P. aeruginosa* strains



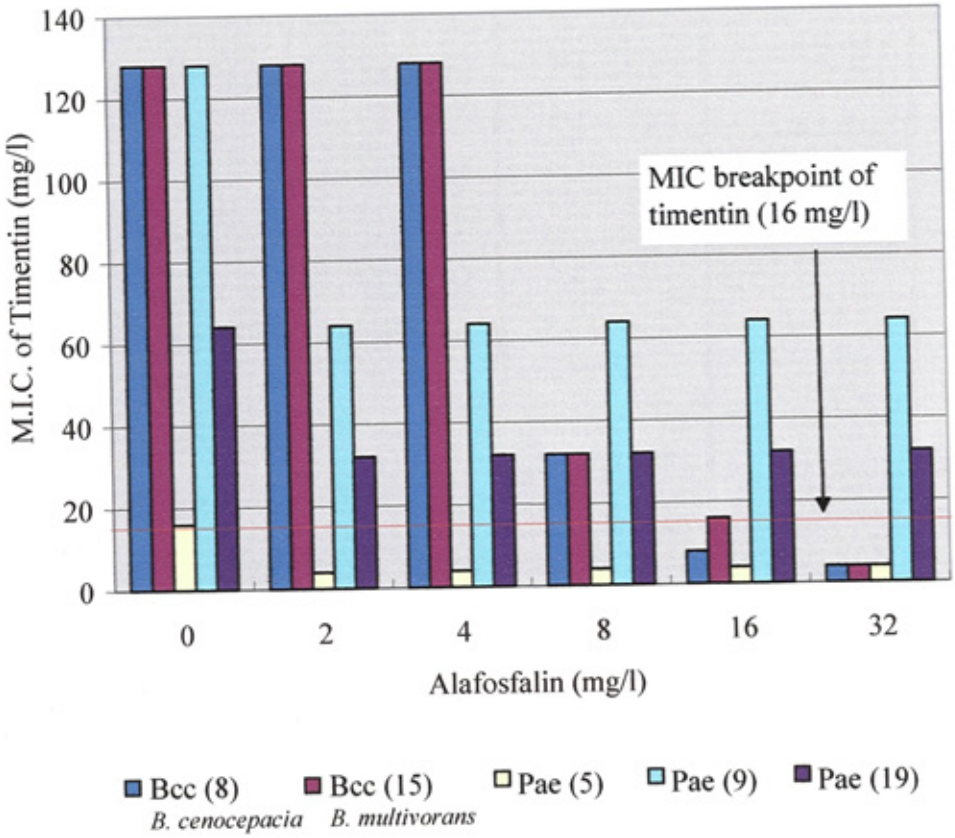
Pae – *P. aeruginosa*

Figure 4.8. Effect of alafosfalin concentration on the M.I.C. of meropenem for Bcc and *P. aeruginosa* strains



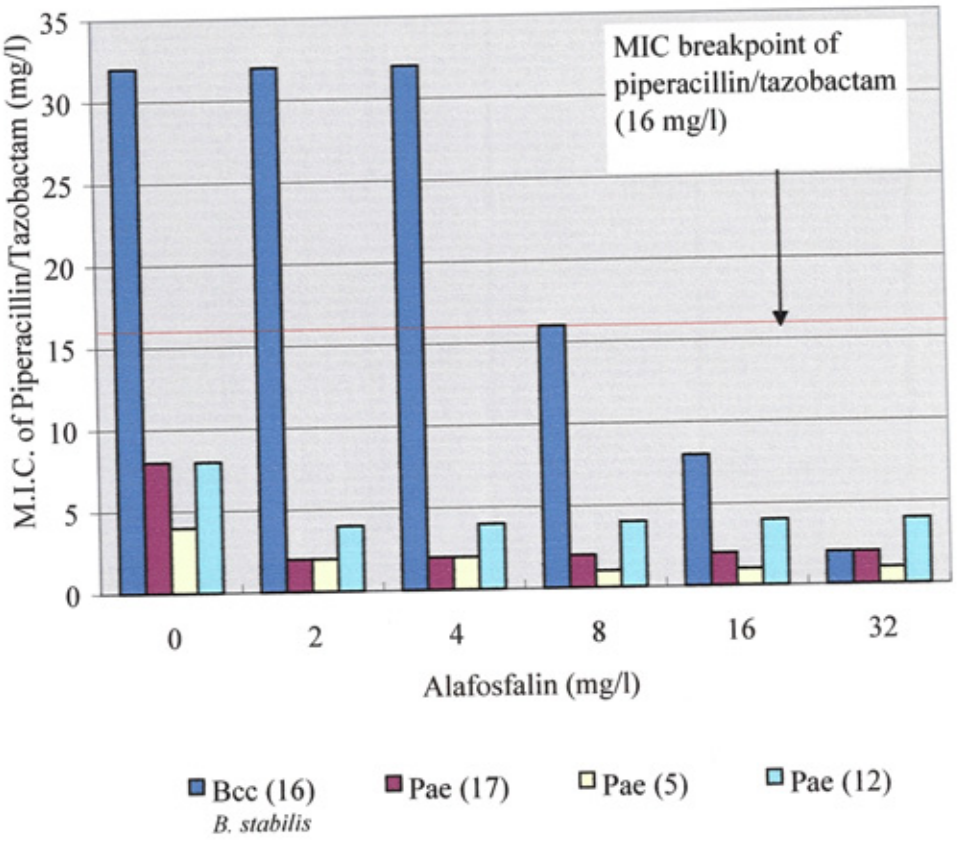
Pae – *P. aeruginosa*

Figure 4.9: Effect of alafosfalin concentration on the M.I.C. of Timentin for Bcc and *P. aeruginosa* strains



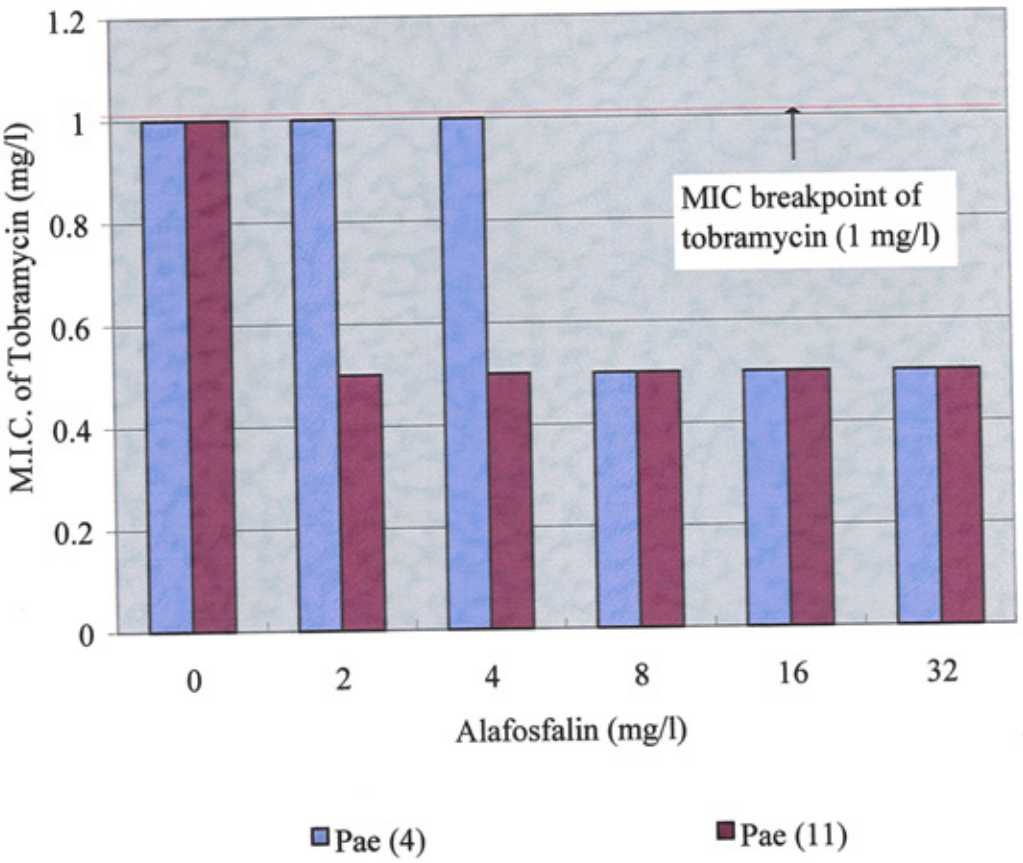
Pae – *P. aeruginosa*

Figure 4.10: Effect of alafosfalin concentration on the M.I.C. of piperacilin/tazobactam for Bcc and *P. aeruginosa* strains



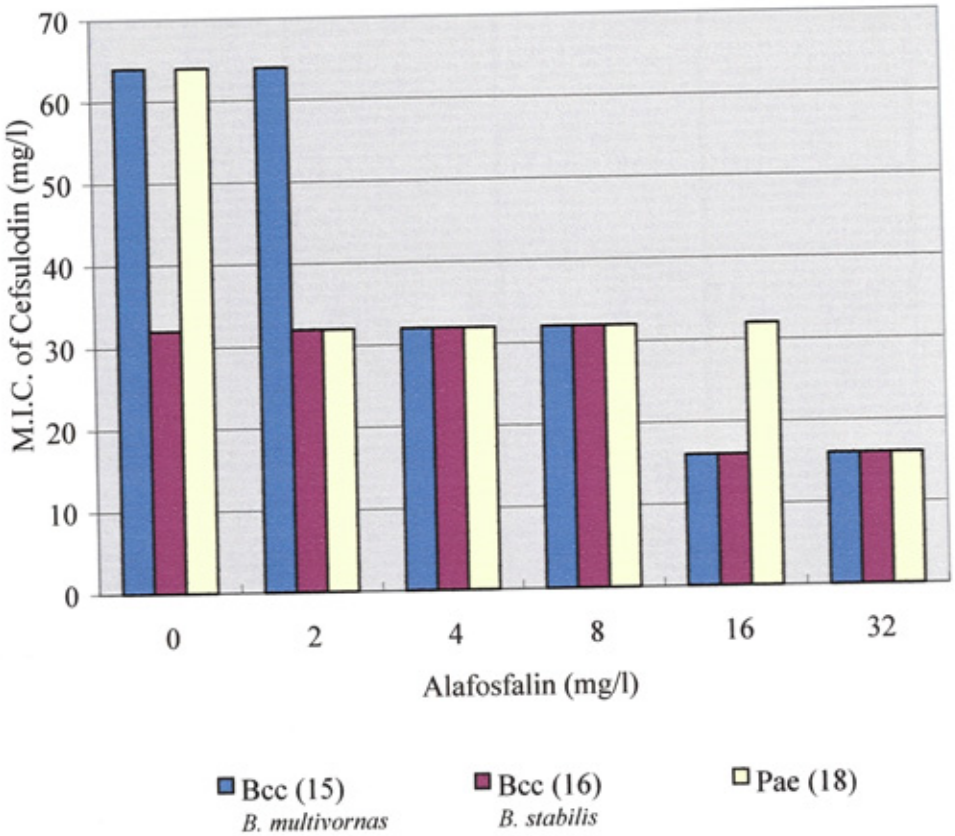
Pae – *P. aeruginosa*

Figure 4.11: Effect of alafosfalin concentration on the M.I.C. of tobramycin for *P. aeruginosa* strains



Pae – *P. aeruginosa*

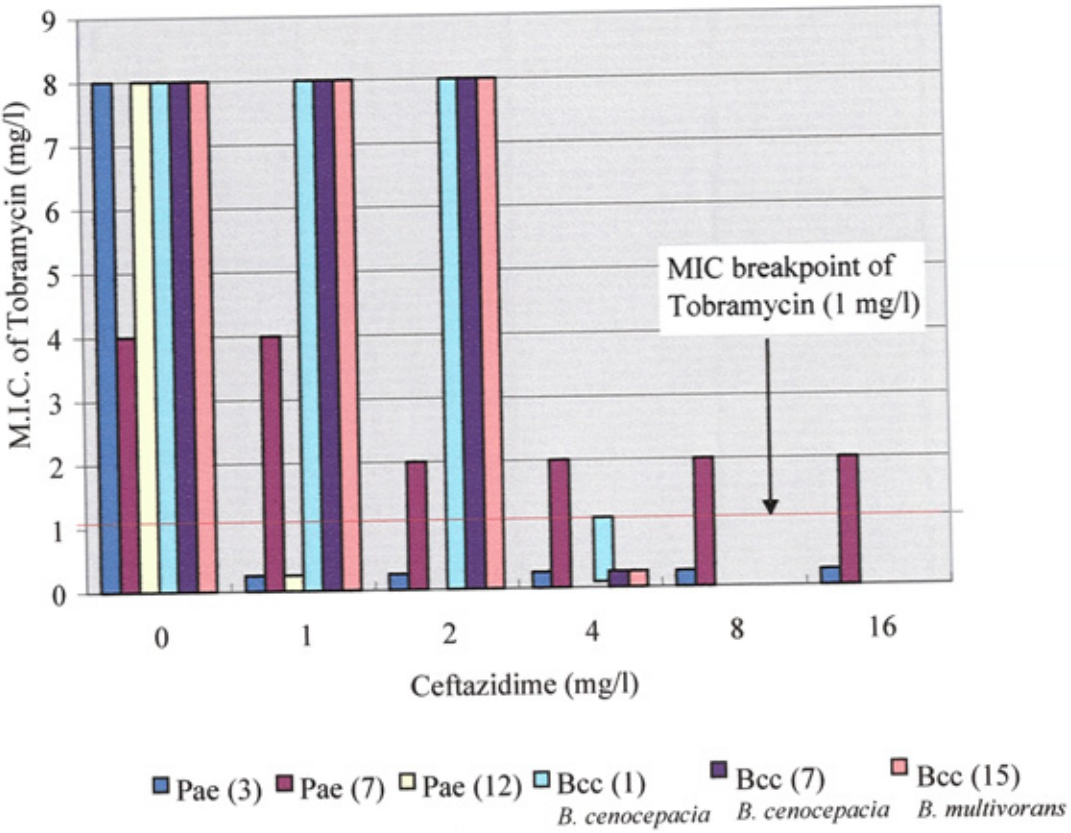
Figure 4.12: Effect of alafosfalin concentration on the M.I.C. of cefsulodin for *P. aeruginosa* and Bcc strains



Pae – *P. aeruginosa*

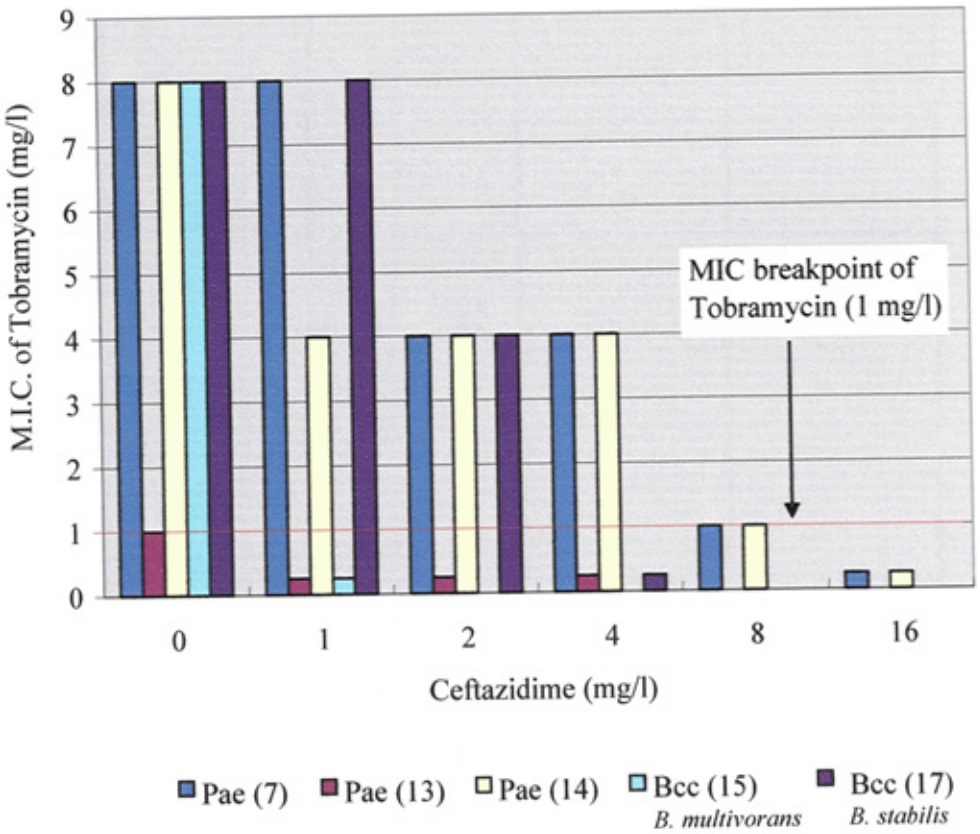
There is no breakpoint value shown for cefsulodin on this graph, against Bcc and *P. aeruginosa* isolates. This is due to the fact that there is no reported breakpoint value by BSAC (Hamilton-Miller, 1999)

Figure 4.13: Effect of ceftazidime concentration on the M.I.C. of tobramycin for *P. aeruginosa* strains



Pae – *P. aeruginosa*

Figure 4.14: Effect of ceftazidime concentration on the M.I.C. of tobramycin for *P. aeruginosa* and Bcc strains in the presence of 32 mg/l alafosfalin.



Pae – *P. aeruginosa*

The most significant reductions are those which lower the MIC of the antibiotic to below its breakpoint concentration. Looking at the combinations which gave the highest levels of synergy, i.e. ceftazidime with alafosfalin for Bcc, and tobramycin with ceftazidime and alafosfalin for *P. aeruginosa*, it can be seen that the MICs of ceftazidime when tested against four Bcc strains, illustrated in Figure 4.6, were reduced to below the breakpoint concentration (8 mg/l), and that the MICs of tobramycin when tested against two isolates of *P. aeruginosa,* illustrated in Figure 4.14, were reduced to below the breakpoint concentration of tobramycin (1 mg/l), without exceeding the breakpoint concentration of ceftazidime. The MIC for the third isolate of *P. aeruginosa* illustrated (isolate no.13) was already below the breakpoint concentration of tobramycin in combination with 32 mg/l alafosfalin but without the addition of ceftazidime. The MIC of ceftazidime and tobramycin, without the 32 mg/l alafosfalin, against this strain was still below the breakpoint without the addition of ceftazidime. Therefore, in this particular isolate, the alafosfalin had no additional effect on the synergy observed.

In contrast, Figure 4.15 illustrates a combination where alafosfalin had no effect on the MIC of the antibiotic in combination, i.e. alafosfalin with ciprofloxacin.

Figure 4.15: Effect of alafosfalin concentration on the M.I.C. of ciprofloxacin for Bcc strains

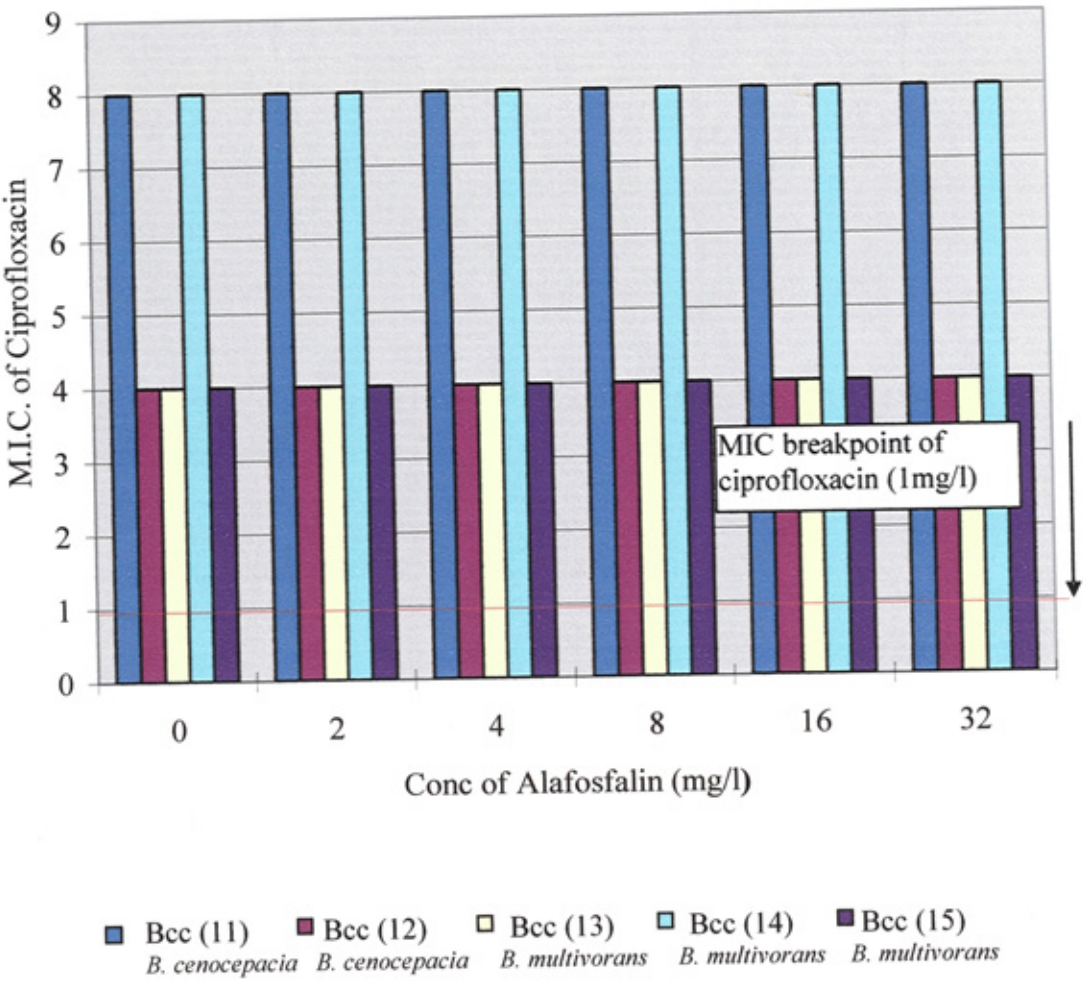
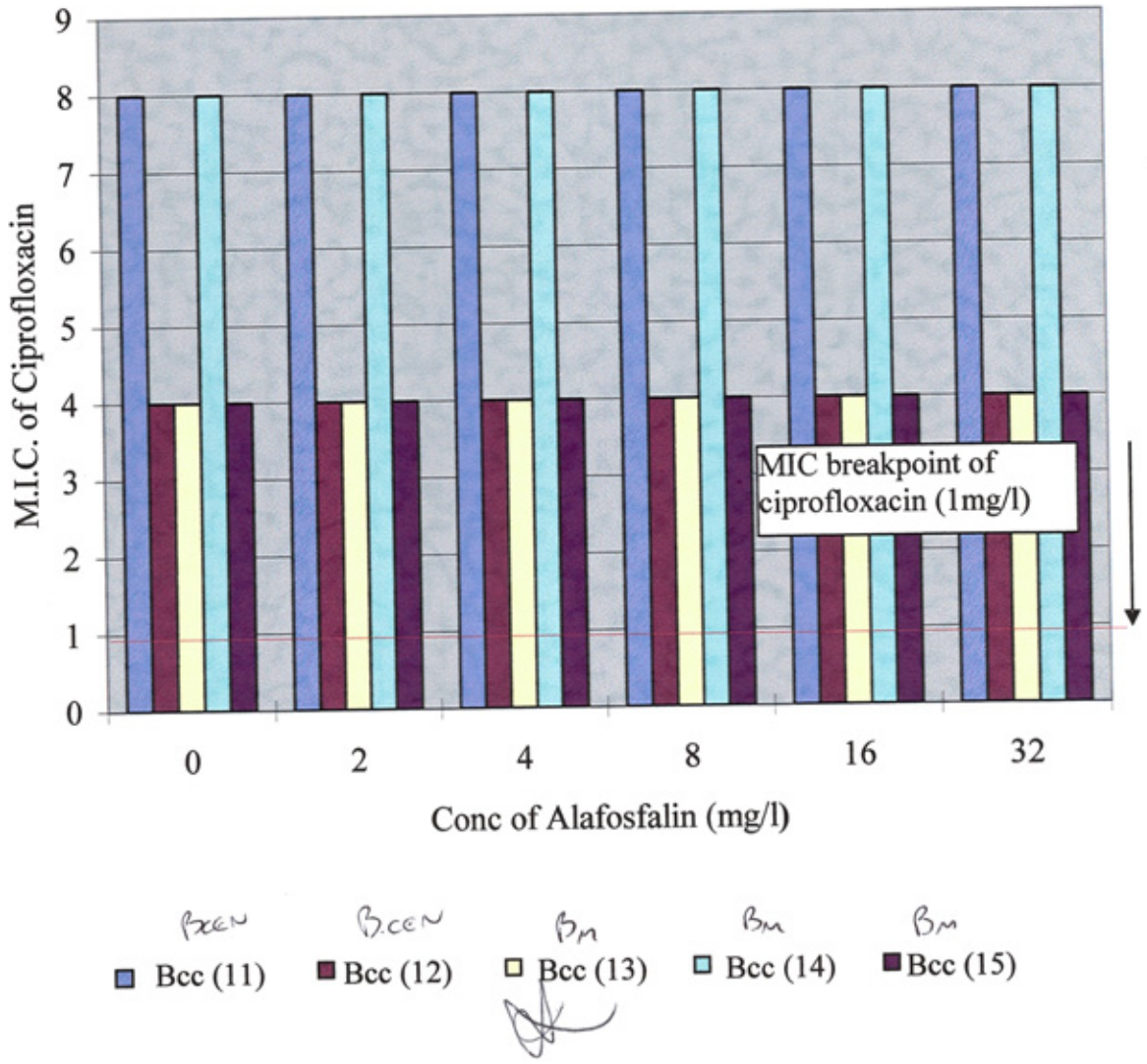


Figure 4.15: Effect of alafosfalin concentration on the M.I.C. of ciprofloxacin for Bcc strains



The reproducibility of the tests were examined to ensure results were reliable (see Table 4.4)

Table 4.4: Reproducibility of synergy tests against Bcc and *P. aeruginosa* strains using the chequerboard technique

Antimicrobial combination	Organism	
	Bcc	*P. aeruginosa*
Ceftazidime/alafosfalin	57.9 (63.2)	84.2 (84.2)
Piperacillin-tazobactam/alafosfalin	94.7 (94.7)	78.9 (84.2)
Timentin/alafosfalin	89.5 (89.5)	84.2 (89.5)
Cefsulodin/alafosfalin	100 (100)	89.5 (89.5)
Aztreonam/alafosfalin	89.5 (100)	84.2 (89.5)
Meropenem/alafosfalin	73.7 (78.9)	78.9 (84.2)
Ciprofloxacin/alafosfalin	100 (100)	100 (100)
Tobramycin/alafosfalin	100 (100)	84.2 (84.2)
Tobramycin/ceftazidime	94.7	94.7
Tobramycin/ceftazidime/alafosfalin	94.7	73.6
Average	**89.5 (90.8)**	**85.3 (88.2)**

These numbers indicate the percentage of strains which showed exact reproducibility. The numbers in brackets indicate the percentage of strains which showed reproducibility but where minor synergy or addition was treated as synergy. With combinations containing tobramycin and ceftazidime, addition could not be treated as synergy as both agents were active against some strains. These figures illustrate that the results achieved are sufficiently reproducible and therefore reliable.

In light of the variation in clinical outcome depending upon which Bcc species is involved in CF patient colonization, Table 4.5 shows the percentage of antibiotic combinations which exerted a synergistic effect in each of the Bcc species tested (genomovars I-V). Figure 4.7 illustrates these percentages.

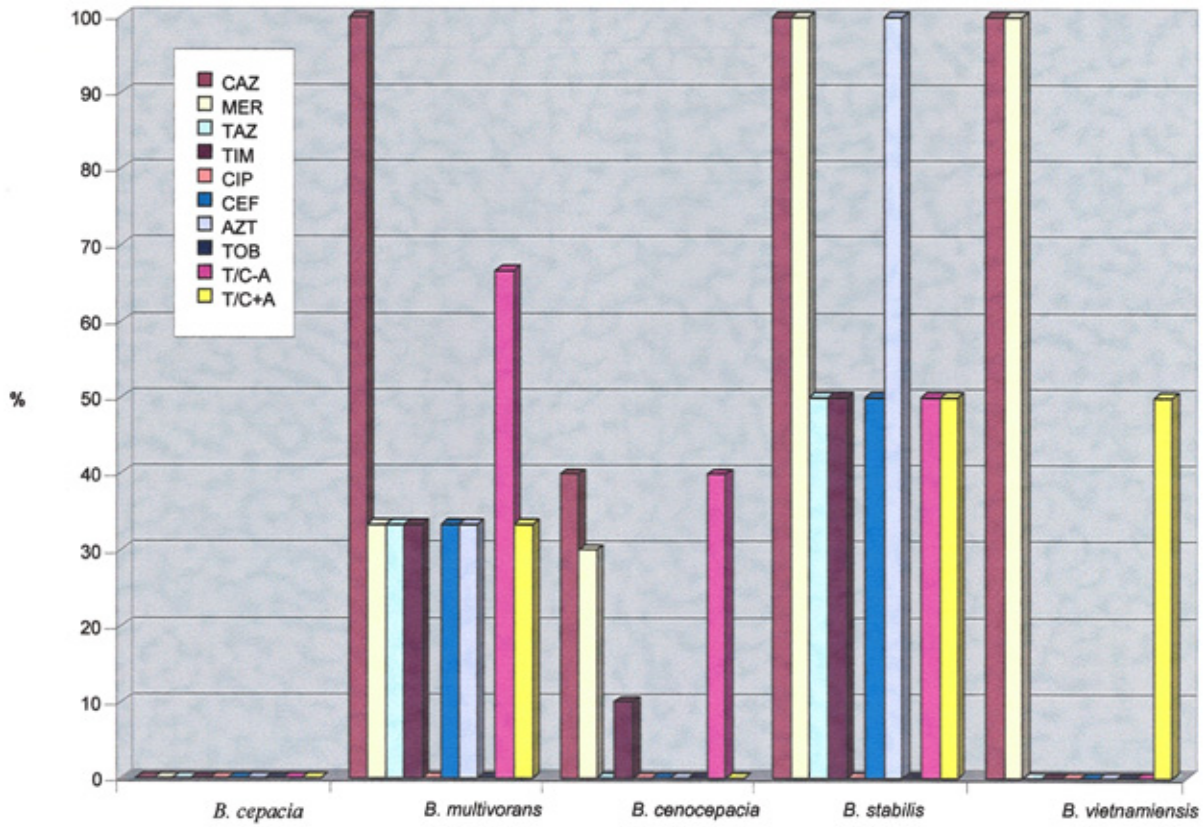
Table 4.5: Percentage of Bcc strains in which alafosfalin/antibiotic combination exerted a synergistic effect.

Bcc sp.	No.	Antimicrobial combination									
		CZ/A	M/A	PT/A	TC/A	CP/A	CF/A	AZ/A	TB/A	TB/CZ	TB/CZ/A
Bce	2	0	0	0	0	0	0	0	0	0	0
Bm	3	100	33	33	33	0	33	33	0	67	33
B.cen	10	40	30	0	10	0	0	0	0	40	0
Bs	2	100	100	50	50	0	50	100	0	50	50
Bv	2	100	100	0	0	0	0	0	0	0	50

Key:
Bce, *B. cepacia*; Bm, *B. multivorans*; Bcen, *B. cenocepacia*; Bs, *B. stabilis*; Bv, *B. multivorans*; A, alafosfalin; CZ, ceftazidime; M, meropenem; PT, piperacillin/tazobactam; TC, ticarcillin/clavulanic acid; CP, ciprofloxacin; CF, cefsulodin; AZ, aztreonam; TB, tobramycin.

Overall, *B. multivorans* and *B. stabilis* showed the greatest level of susceptibility to the antibiotic combinations tested. Ceftazidime and alafosfalin in combination exerted a synergystic effect on all *B. multivorans, B. stabilis* and *B. vietnamiensis* strains, while a combination of meropenem and alafosfalin exhibited synergy against all *B. stabilis* and *B. vietnamiensis* strains. *B. cenocepacia* strains showed a poor response to most combinations, although some strains were susceptible to some of the combinations. For example, alafosfalin with ceftazidime, and tobramycin with ceftazidime exerted a synergistic effect on 40 % of *B. cenocepacia* strains. The greatest degree of synergy was observed with ceftazidime or meropenem combined with alafosfalin against *B. vietnamiensis* strains. None of the antibiotic combinations tested demonstrated a synergistic effect on either of the two *B. cepacia* strains included in the study.

Figure 4.16: Graph illustrating percentage of Bcc strains in which alafosfalin/antibiotic combinations exerted a synergistic effect.



Key:
CAZ, ceftazidime; MER, meropenem; TAZ, piperacillin/tazobactam; TIM, ticarcillin/clavulanic acid; CIP, ciprofloxacin; CEF, cefsulodin; AZT, aztreonam; TOB, tobramycin; T/C-A, tobramycin with ceftazidime; T/C+A, tobramycin with ceftazidime and alafosfalin.

Discussion

In the case of CF lung disease, complete elimination of the organism is usually impossible because of the complex interrelationships between host defenses in patients with CF and these microbes (Aaron *et al.*, 2000). The objectives of treatment in CF are therefore not long-term eradication of the organism but rather control of infection in order to minimize inflammation and damage and thus slow the decline of pulmonary function.

Controlling infection and minimizing inflammation can be achieved by antimicrobial therapy that reduces the numbers of organisms (Regelman *et al.*, 1990; Smith *et al.*, 1999). In addition to their bactericidal activity, antibiotics have also been shown to suppress bacterial synthesis of pathogenic factors that are potent inciters of inflammation within CF airways, and antibiotics can exert antioxidant effects by neutralizing myeloperoxidase release from polymorphonuclear cells in CF sputum (Cantin and Woods, 1993). Antimicrobial management in cystic fibrosis patients remains a complex problem because of the chronic course of the disease associated with repeated episodes of infections. It is therefore not surprising that many of the bacteria causing these infections eventually acquire resistance to many commonly used antibiotics. *In vitro* demonstration of synergy could be important in selecting therapy and guiding treatment, and helps in avoiding antagonistic combinations. To predict a synergistic combination to be clinically beneficial, it is important for the concentration of the antipseudomonal drug in the combination to be within its clinically achievable concentration range. The British Society for Antimicrobial Chemotherapy (BSAC) has

published standardized breakpoints for *P. aeruginosa* for various antibiotics to define susceptibility (Hamilton-Miller, 1999; Andrews, 2001).

Previous synergy studies incorporating alafosfalin

Although there has been very little interest in the compound alafosfalin in the last 20 years, previous studies have highlighted the synergistic effects observed between alafosfalin and cell-wall acting antibiotics, in particular ß-lactams, against various bacterial species, including *P. aeruginosa* (Allen *et al.*, 1980; Atherton *et al.*, 1981; Arisawa *et al.*, 1982; Maruyama *et al.*, 1979). Atherton *et al,* (1981) investigated the antibacterial properties of alafosfalin combined with cephalexin, mecillinam and ampicillin and demonstrated that potentiation could be seen in wide range of genera, however, neither *P. aeruginosa* nor Bcc strains were tested in this study. Maruyama *et al.* (1979) evaluated alafosfalin for *in vitro* antibacterial activity and for synergism with ß-lactams ampicillin and cephazolin. They demonstrated that potentiation was found in a wide range of genera, particularly Gram negatives such as *E. coli*, *Serratia* sp. and *Klebsiella* sp. This study included *P. aeruginosa* strains and found alafosfalin alone was inactive against *P. aeruginosa* but showed minor synergy when in combination with these ß-lactams antibiotics. Arisawa *et al.* (1982) reported a synergistic effect on three *P. aeruginosa* strains when alafosfalin was combined with cefsulodin, even though they too found *P. aeruginosa* to be resistant to alafosfalin alone. We demonstrated a synergistic effect in 11 % of strains of *P. aeruginosa* for this same combination. Our results confirm the findings of these early studies that *P. aeruginosa* isolates are resistant to alafosfalin if this antibiotic is used alone, but can show some susceptibility if

alafosfalin is in combination with a ß-lactam antibiotic. We also found this to be applicable in a number of Bcc strains.

Previous synergy studies on Bcc and *P. aeruginosa* strains compared to combinations involving alafosfalin

Relatively high rates of synergy (in >50 % of isolates) in both Bcc and *P. aeruginosa* strains have been reported for combinations of various antibiotics, in particular, aminoglycosides with ß-lactam drugs (Bosso *et al.*, 1987; Aaron *et al.* 2000; Hoiby, 2002). In our study, alafosfalin combined with the ß-lactam antibiotic ceftazidime was found to be the most effective combination against the Bcc isolates tested, as synergy was observed in the highest number of Bcc strains with this combination. Synergy was only observed in 11 % of *P. aeruginosa* isolates with this combination. Alafosfalin was successful in reducing the MIC of ceftazidime to below its breakpoint concentration (8 mg/l) in a number of Bcc isolates. Tobramycin with ceftazidime was also found to be an effective combination against Bcc strains, as well as *P. aeruginosa* isolates. Of the *P. aeruginosa* isolates tested, tobramycin and ceftazidime with alafosfalin was found to be the most effective combination, having a synergistic effect on 58 % of the strains tested (32 % with FIC < 0.5). The MIC of tobramycin was reduced to below its breakpoint concentration (1 mg/l) in a number of Bcc and *P. aeruginosa* isolates. This triple combination was synergistic in a much lower percentage of Bcc strains (16 %).

Aaron *et al.* (2000) found that tobramycin with ceftazidime was the most effective double antibiotic combination that did not contain meropenem, against Bcc strains and was bactericidal against 52 % of isolates. This is higher than the 37 % of Bcc isolates in

which synergism was demonstrated in our study, however, Aaron *et al.* used the Multiple Combination Bactericidal Antibiotic Testing (MCBT) technique, not a chequerboard, so the results cannot be directly compared. Aaron *et al.* also found antagonism to be occurring between meropenem and tobramycin in 22 % of Bcc strains, and between ceftazidime and tobramycin in 18 % of isolates. Lang *et al.* (2000) observed antagonism in 13 % of meropenem and ceftazidime combinations, and 2 % of tobramycin and ceftazidime combinations against *P. aeruginosa* isolates. In contrast to this, the present study did not demonstrate antagonism between any of the double antibiotic combinations tested, although an antagonistic effect occurred when tobramycin was added to the double combination of alafosfalin with ceftazidime in Bcc strains, as the number of isolates in which synergy was observed decreased from 42 % to 16 %. Owens *et al.* (1997) observed synergistic activity in combinations of tobramycin in combination with either piperacillin/tazobactam or ticarcillin/clavulanic acid in *P. aeruginosa* isolates. However, in contrast to the present study which found tobramycin in combination with ceftazidime to be synergistic against a number of *P. aeruginosa* isolates (42 %), Owens *et al.* found that although tobramycin with piperacillin/tazobactam showed synergy against these isolates, tobramycin with ceftazidime had no effect. In agreement with the present study, Weiss and Lapointe (1995) studied the effects of tobramycin in combination with various ß-lactam antibiotics against *P. aeruginosa* and found tobramycin with ceftazidime to be the most effective combination, demonstrating synergy against 39 % of isolates. The triple combination of tobramycin and ceftazidime with alafosfalin was synergistic in a greater number of *P. aeruginosa* isolates tested (58 %).

Lang *et al.* (2000) carried out a MCBT study on *P. aeruginosa* isolates. They demonstrated that the bactericidal activity of antibiotics was significantly enhanced by the addition of tobramycin. This is in agreement with our study which demonstrated that the addition of tobramycin to the combination of ceftazidime with alafosfalin, against *P. aeruginosa* isolates, increased the synergy observed from 11 % to 58 %. However, the addition of tobramycin to this combination against Bcc strains decreased the figures from 42 % to 16 %.

Aaron *et al.* (2000) found meropenem to be by far the most effective antibiotic in combination with various other antibiotics against Bcc strains. They found that among all the Bcc isolates, a double antibiotic combination that contained meropenem was bactericidal in 62 % cases. They found meropenem with ceftazidime and meropenem with tobramycin, bactericidal against 73 % and 64 % isolates respectively. Meropenem together with alafosfalin demonstrated synergy against a high proportion of the Bcc isolates (32 %). Although the MIC of meropenem was below its breakpoint concentration for the isolates without the addition of alafosfalin, the two antibiotics in combination are likely to be more effective than meropenem alone, as resistant mutations would be less likely to appear. Alafosfalin combined with aztreonam or meropenem or piperacillin/tazobactam demonstrated a synergistic effect in a number of *P. aeruginosa* isolates and again, the MICs of the ß-lactams were reduced by addition of alafosfalin, to below their breakpoint concentrations.

A number of *P. aeruginosa* isolates showed synergy with the double combinations of ticarcillin/clavulanic acid and tobramycin each with alafosfalin. However, the addition of alafosfalin did not reduce the MIC of ticarcillin/clavulanic acid to below its

breakpoint concentration in these strains, and the MIC of tobramycin was already below its breakpoint concentration without the presence of any alafosfalin. Cefsulodin in combination with alafosfalin demonstrated a synergistic effect in a small number of *P. aeruginosa* and Bcc isolates.

Ciprofloxacin has been shown to work synergistically with a variety of other antimicrobial agents, mainly ß-lactams, against *P. aeruginosa.* For example, Bonacorsi *et al* (1999) found ciprofloxacin and meropenem to be a synergystic combination. Ciprofloxacin and fosfomycin have been shown to demonstrate synergy against 16-17 % of *P. aeruginosa* isolates (Vincent *et al.,* 1988). Lang *et al.* (2000) found ciprofloxacin with meropenem to be the most effective double antibiotic combination, excluding those with tobramycin, against *P. aeruginosa* isolates (synergistic against 85 % of isolates). *In vitro* studies have demonstrated synergistic effects against Bcc strains for meropenem plus ciprofloxacin and ceftazidime plus ciprofloxacin (Banjeree and Stableforth, 2000). In contrast to this, there was no synergy observed between ciprofloxacin and alafosfalin against any Bcc or *P. aeruginosa* strains. More in keeping with our results, a study by Isenburg *et al.* (1999) showed ciprofloxacin and ß-lactam combinations showing synergy against none, or only a small fraction (7-10 %) of *P. aeruginosa* and Bcc isolates. Aaron *et al.* (2000) observed antagonism in 11 % Bcc isolates tested with ciprofloxacin with ceftazidime.

Triple antibiotic combinations

The MCBT studies performed by Aaron *et al.* (2000) and Lang *et al.* (2000) compared the value of double and triple antibiotic combinations against *P. aeruginosa* and Bcc isolates. Aaron *et al.* (2000) found that out of 119 "*B. cepacia*" isolates, 50 % of isolates were

resistant to all single antibiotics tested, 8 % were resistant to all two-drug combinations, but all were inhibited by at least one bactericidal triple-drug combination. They concluded that triple combinations are more likely than double and single antibiotic combinations to be bactericidal against "*B. cepacia*" *in vitro*. By studying 75 *P. aeruginosa* isolates, Lang *et al.* (2000) found that addition of a second antibiotic significantly improved bactericidal activity, i.e. double combinations were the most effective, and that addition of a third antibiotic may be unnecessary. Adding a third antibiotic did not significantly improve inhibition *in vitro*.

One triple combination was set up to include alafosfalin in this study, that being alafosfalin in combination with both tobramycin and ceftazidime. Although a direct comparison cannot be made with these two previous studies due to the different techniques adopted, it is interesting to note that in contrast to the results of Lang *et al.* (2000) and Aaron *et al.* (2000), our study demonstrated the triple combination to be more effective against *P. aeruginosa* strains than the two individual double combinations, with synergy observed in 58 % of strains with the triple combination compared to 42 % of strains with tobramycin and caftazidime, and to 11 % of strains with alafosfalin and ceftazidime. Indeed, this triple combination was synergistic in a higher percentage of strains than for any of the double combinations tested. We also demonstrated, again in contrast to these two studies, that the triple combination (for which synergy was observed in 16 % strains) was less effective than the two individual double combinations of tobramycin with ceftazidime (37 % of strains) and alafosfalin with ceftazidime (42 % of strains).

Synergy between alafosfalin and ß-lactams

Most effective antibiotics demonstrating synergy in combination with alafosfalin were tobramycin and ceftazidime together, aztreonam, and meropenem for *P. aeruginosa* strains, and ceftazidime and meropenem in Bcc strains. These antibiotics are all ß-lactams excluding tobramycin, but tobramycin with alafosfalin only demonstrated synergy in a high number of isolates when in combination with the ß-lactam antibiotic ceftazidime. All ß-lactam antibiotics, and monobactams target the cell wall by interfering with cross-linkages of peptidoglycan molecules. In low concentrations of these antibiotics septum formation is inhibited, when high concentrations of the drug are present, osmotic lysis of bacteria with defective cell walls occurs. The different mechanisms of action on the bacterial cell wall of alafosfalin and ß-lactams in inhibiting bacterial peptidoglycan synthesis suggests that a "multiblockade" cell wall synthesis phenomenon could be assisting in synergy. The synergy observed with alafosfalin and certain ß-lactam antibiotics may involve a double block on the availability of D-alanine, since inhibitors of cell wall transpeptidase and carboxypeptidase would prevent any re-utilization of D-alanine released during peptidoglycan cross-linking (Ghuysen and Shockman, 1973). Whether this is due to sequential blockade of the racemase and synthetase enzymes, or to double inhibition of the racemase is unknown.

In combination with alafosfalin, piperacillin/tazobactam and ticarcillin/clavulanic acid demonstrated fairly high percentages of synergy in *P. aeruginosa* isolates, but not in Bcc isolates. This suggests that the inactivation of ß-lactamases produced by the *P. aeruginosa* isolates allows these antibiotics to act synergistically with alafosfalin. Piperacillin/tazobactam with alafosfalin gave a greater number of isolates with FIC <

0.5, compared to ticarcillin/clavulanic acid. This could be because tazobactam is a ß-lactamase inhibitor with greater activity than clavulanic acid against many cephalosporinases.

Atherton *et al.* (1981) noted that both mecillinam and cephalexin can exhibit penicillin-binding protein (PBP) profiles of an incomplete nature. Mecillinam binds solely to PBP2 of *E.coli*, whereas the binding of cephalexin to PBP3 results in the growth of filamentous cell forms over a wide range of antibiotic concentrations (Spratt and Pardee, 1975). These results suggest that ß-lactam antibiotics showing incomplete binding profiles may be especially susceptible to synergy with alafosfalin. Tobramycin was evaluated with alafosfalin as aminoglycosides are an effective drug to treat pseudomonal infections. This antibiotic showed no synergy with alafosfalin against any Bcc isolates, and in a low percentage of *P. aeruginosa* isolates. As an aminoglycoside, tobramycin interferes with mRNA attachment to the ribosome. As a non-cell wall acting antimicrobial, the lack of synergy observed when in combination with alafosfalin was therefore not unexpected.

As well as cell wall-acting antibiotics, ciprofloxacin was tested additionally as another example of a non-cell wall acting antimicrobial. This quinolone targets DNA replication, and attacks a wide range of Gram negative bacilli. *P. aeruginosa* infections have been successfully treated, including some infections in CF patients (Aronoff and Klinger, 1984; Bosso *et al.*, 1990). It has already been discussed that ciprofloxacin in combination with alafosfalin showed no synergistic effect on any *P. aeruginosa* or Bcc isolates. This lack of synergy between alafosfalin and a non-cell wall acting antibiotic

suggests that a "multiblockade" cell wall synthesis phenomenon could be assisting in alafosfalin/antibiotic combinations.

Synergy testing *in vitro*

One problem with synergy testing, and antimicrobial susceptibility testing in general is that *in vitro* methods are intended to simplify an intricate process that occurs in the human body. In humans, drug concentrations, bacterial inoculum size, and host defense contributions are continually changing in quantity and potentially in quality over time, and the ability to capture the dynamics of this complex organism-host relationship is beyond current technology. We therefore depend on currently available methods for synergy determination to assess the complex nature of these drug relationships; as a result, these data may be useful only when the limitations (e.g. variability in interpretive criteria and lack of reproducibility) of *in vitro* testing are respected (Owens *et al.*, 1997). Despite these limitations, the observation of synergism *in vitro* is generally considered beneficial in the treatment of infection. There are many different ways to test for synergistic combinations, and when comparisons are made between results, it must be remembered that results derived from different methodologies cannot be directly compared.

As mentioned in the introduction, chequerboards and time kill studies are commonly adopted to study antibiotic-antibiotic interactions. However, a number of other methods are available. An alternative method for synergy testing is Multiple Combination Bacterial Antibiotic Testing (MCBT) (Aaron *et al.*, 2000., Lang *et al.*, 2000). This technique uses microtitre technology with a bactericidal endpoint. Bactericidal activity

was defined as the absence of growth on subculture (from a nonturbid microtitre well) of an organism in the presence of antibiotics. The inoculum used is 10^5 cfu/ml, lower than the bacterial load in CF sputum, and the antibiotic concentrations used reflect peak concentrations. Aaron *et al.* (2000) developed this technique for rapid *in vitro* testing of multiple antibiotic combinations for Bcc strains.

This technique has advantages and disadvantages compared with the chequerboard and time kill studies. MCBT allows multiresistant bacteria to be tested for susceptibility against numerous combinations of antibiotics, single, double or triple combinations, and the results of the test are available within 48 to 72 hrs after bacterial species isolation. MCBT technique is not a method designed to detect antibacterial-antibacterial interaction but rather an index of total bactericidal activity. However, there is little reproducibility data and the clinical correlates are weak. Bacterial kill curves are bactericidal and the measure of viable counts reasonably accurate. However, the tests are time consuming and the interpretative criteria arbitrary (MacGowan *et al.*, 2003). With bacterial kill curves there are few clinical correlates and reproducibility is often not assessed (MacGowan, 2003). The chequerboard technique has been reported to be unreproducible (Rand *et al.*, 1993), but our reproducibility data shows this not to be the case in this study. Chequerboard tests are also time consuming to prepare, however, the results are usually available within 48 hrs.

If sputum is cultured at the time of a CF pulmonary exacerbation, the MCBT test results can be used to select appropriate bactericidal combinations and discontinue non-bactericidal antibiotic therapy within days of a patient's exacerbation. In addition, MCBT susceptibilities can also be collected when the patient is clinically stable, to

allow clinicians to decide on appropriate antibiotic combination therapy in advance of the patient's next pulmonary exacerbation. The test is simple to set up. However, only one concentration is generally used for each antibiotic, it would be very difficult and time consuming to set up combinations using numerous concentrations. Bacterial kill curves can be used but usually only one or two concentrations of each drug are employed (MacGowan, 2003). It would seem that MCBT is very much suited to a routine laboratory situation, where a number of antibiotics require screening in as short a time possible. Such a standardized methodology for routine antimicrobial susceptibility testing is highly desirable. This is very important as reporting of a multidrug-resistant *P. aeruginosa* strain can affect hospital infection control and isolation policies (Burns *et al.*, 2000). It is also important for tracking of resistance patterns in the CF population, especially as new therapeutic agents are developed and traditional antibiotics are delivered to more patients via aerosol (Shawar *et al.*, 1999). The chequerboard technique is probably more suited to research situation where a range of antibiotic concentrations are more likely to be required, or in a clinical setting to find out more detailed information about drug interactions between two antibiotics being taken by a patient.

One problem with chequerboards is that FIC indices are highly dependent on the dilution series, as shown by Horrevorts *et al.* (1987) and as such, chequerboard titrations would best be carried out with serial dilutions in which the intervals between consecutive concentrations would be constant (Berenbaum, 1983). However, it is difficult to prepare such serial dilutions, and, in addition, an unworkable high number of concentrations would be required to cover a sufficiently wide range. While tests such as those described in the present study cannot reasonably be expected to predict clinical outcome (which

depends on many other factors as well) the results of such studies may be helpful in guiding therapy.

The E test has recently been used to calculate FIC indices as the basis of studying the pharmacodynamics of combination therapy (White *et al.,* 1996; Den Hollander *et al.,* 1998; Manno *et al.* 2003) and the results show good correlation with chequerboards. Manno *et al.* (2003) examined the use of the E test to assess synergy of antibiotic combinations against isolates of Bcc strains from patients with cystic fibrosis. They compared the E test to the chequerboard method using various combinations of antimicrobial agents. Agreement between the E test and the chequerboard method was 90 %. The results suggest that the E-test is a valuable and practical method to be considered for improving the identification of possible therapeutic options in CF patients infected with Bcc strains.

If time kill studies can be combined with chequerboards, then much more detailed kinetic information can be generated (Mackay *et al.*, 2000). Burns *et al.* (2000) compared agar diffusion methodologies for antimicrobial susceptibility testing of *P. aeruginosa* isolates from CF patients. They examined the correlation of disk diffusion and E test results with a reference broth microdilution method. Overall, both agar diffusion methods appeared to be broadly acceptable for routine clinical use in susceptibility testing of CF isolates of *P. aeruginosa,* both mucoid and non mucoid isolates. Breakpoint combination sensitivity testing has been evaluated for studying synergy between *P. aeruginosa* and Bcc isolates (Tunney and Scott, 2004). The results were found to correlate well with chequerboard technique, and is simple and convenient to use in a routine laboratory.

Use of alafosfalin in the clinical setting

Alafosfalin was originally developed with treatment of urinary infections in mind, but showed low, but dose dependent urinary recovery, varying from 6-17 % after oral administration due to saturable tubular reabsorption, and considerable decrease of urinary concentrations with impaired renal function (Neuman, 1984). It was thought also that its spectrum, which is well suited for bacteria that cause gastro-intestinal infections, such as *E. coli, Shigella* sp. and *Salmonella* sp. could make it a useful alternative to trimethoprim-sulphonamide combinations in the treatment of these infections (Westmacott *et al.*, 1981). As a treatment for lung colonization, in combination therapy, the problems of low urine recovery are irrelevant.

The incidence of resistance to alafosfalin is low (Allen *et al.*, 1979a; Maruyama *et al.*, 1979; Atherton *et al.*, 1981). Resistance to alafosfalin generally arises from the selective growth of a subpopulation unable to transport phosphonodipeptides at appreciable rates (Atherton *et al.*, 1981). However, suitable combinations of alafosfalin and cephalexin were shown to be very effective in controlling development of strains which are resistant to either antibiotic alone, even under the excessive selective pressure encountered in the *in vitro* methods employed. The inhibition of resistance development is a direct consequence of the lack of cross-resistance between alafosfalin and cephalexin (Maruyama *et al.*, 1979). This suggests that if used in combination with additional antibiotics, resistance may not be a problem when using alafosfalin.

A number of antibiotics, including aminoglycosides, cephalosporins and ß-lactams demonstrate altered pharmacokinetics in patients with CF. Reasons for this include

increased renal clearance, liver enzyme induction and greater volume of distribution as a result of a malnutrition-related increase in amount of lean tissue per kilogram bodyweight (Banjeree *et al.*, 2000). This means that higher doses of antibiotics must be given to patients with CF, which in turn increases concerns about adverse effects such as toxicity e.g. nephrotoxicity (Banjeree and Stableforth, 2000). This potential problem would have to be investigated if alafosfalin was to be considered as treatment for CF patients.

Although regular courses of intravenous antibiotics have improved survival of CF sufferers by reducing sputum load and maintaining pulmonary function, they interfere with the activities of daily living. As alafosfalin is a small and soluble peptide mimetic, its possible use in nebulised form could be considered. The use of nebulised antibiotics has increased following reports that early treatment can prevent or delay the onset of chronic pseudomonal infection and improve lung function (Touw *et al.*, 1995; Valerius *et al.*, 1991; Hodson *et al.*, 2002; Webb and Dodd, 1997). Overall, about 10 % of a nebulised drug is delivered to the lungs (Le Conte *et al.* (1993). The prescription of nebulised antibiotics for domiciliary use by patients with CF is increasing (Webb and Dodd, 1997; Ryan *et al.*, 2000). The polymyxin antibiotic colistin and the aminoglycosides tobramycin and gentamicin are the most commonly prescribed nebulised antibiotics (Stead *et al.*, 1987; Hodson *et al.*, 2002; Ramsey *et al.*, 1993; Webb and Dodd, 1997). Nebulised antibiotics do not appear to cause renal or nephrototoxicity, and although resistance does occur, it is often intermittent and not related to clinical deterioration (Webb and Dodd, 1997; Banjeree and Stableforth, 2000). Therapy with an intravenous drug and alafosfalin in the aerosolized form could

be a future treatment regimen for acute exacerbations. Pharmaceutical firms would have to decide on the technical feasibility and potential benefits of such a preparation.

Biofilms

One of the reasons for the difficulty in clearing *P. aeruginosa* and Bcc infections in CF patients is the sticky nature of the biofilm (Mathee *et al.*, 1999; Hoiby *et al.*, 2002). The chronic *P. aeruginosa* lung infection in CF is a biofilm, a layer of glycocalyx formed of the various exopolysaccharides secreted by *P. aeruginosa* (Lawrence, 2002). Biofilms are characterized by (i) the mucoid phenotype producing an abundance of alginate *in vitro* and *in vivo*, (ii) microcolonies surrounded by alginate in sputum and in post-mortem investigations and bacteria staying on the surface of the airways as an endobronchiolitis without spreading to the blood or to other organs, (iii) high levels of antibodies against alginate and other *P. aeruginosa* antigens, and (iv) resistance to the patients defence mechanisms and to antibiotic treatment as the biofilm prevents antibiotic penetration and protects the bacteria present within it (Hoiby *et al.*, 2002). Standard laboratory techniques used to determine antibiotic susceptibility of planktonic-growing bacteria cannot predict the possibility of eradication of bacteria growing in biofilms (Domingue *et al.*, 1994). The increased resistance of biofilm-growing bacteria means that antibacterial therapy usually fails to eradicate the bacteria in the biofilm, although the standard laboratory susceptibility tests demonstrate sensitivity to the antibiotics used. This must be considered when testing for synergistic antibiotic combinations *in vitro* such as in our study. Al-Bakri *et al.* (2004) looked at the immigration and emigration of *B. cepacia* and *P. aeruginosa* between and within mixed biofilm communities. They found that colonization of a surface with one bacterial species confers colonization resistance towards the other species.

Alternative antimicrobial synergy testing and treatment for Bcc and *P. aeruginosa* strains

In a study by Fung-Tomc *et al.* (2002), the antibacterial activity of the novel des-fluoro(6)quinolone garenoxacin with non-quinolones was examined against *P. aeruginosa* and "*B. cepacia*". Synergy was observed with garenoxacin plus aztreonam and piperacillin/tazobactam in 5 out of 8 strains of *P. aeruginosa*, and with ceftazidime or aztreonam with garenoxacin in 5 out of 6 strains of "*B. cepacia*". Davis *et al.* (2003) looked at another novel fluoroquinolone, gatifloxacin, in combination with a number of antibiotics including meropenem and piperacillin and found 50-70 % of all drug combinations synergistic against *P. aeruginosa.* Indifference was noted for most *"B. cepacia"* isolates. These figures are significant and illustrate the importance of looking for synergistic combinations against pseudomonal infections using new antimicrobials, as well as more established compounds, such as alafosfalin, which have as yet been little investigated in the CF setting. Garenoxacin and gatifloxacin are unlikely to have a synergistic effect against organisms when in combination with afosfalin, as the quinolones selectively inhibit bacterial DNA gyrase enzymes which then prevent bacterial DNA from being produced, rather than acting on the cell wall.

Alkawash *et al.* (1999) looked at the effect of human lactoferrin on the MICs of doxycycline and rifampicin for Bcc and *P. aeruginosa* strains. The presence of lactoferrin at the concentration found in CF sputum (0.9 g/l) reduced MICs and MBCs of doxycycline for Bcc and *P. aeruginosa* strains. Rifampicin MICs for Bcc strains were also reduced by lactoferrin and for some strains MBCs were reduced. These findings suggest new therapeutic approaches to infections and question the relevance of standard sensitivity tests

for CF pathogens. Addition of lactoferrin to media for the routine sensitivity testing of CF isolates might give more relevant results. It would be of interest to repeat the alafosfalin-antibiotic combinations used in this study against Bcc and *P. aeruginosa* strains in the presence of lactoferrin to see if synergy would occur in a greater number of isolates.

Other novel molecules are under investigation for there potential use in antimicrobial therapy. Designed antimicrobial peptides (DAPs), for example, are laboratory synthesized peptide antibiotics that demonstrate a wide spectrum of antibacterial activity (Schwab *et al.*, 1999). DAPs were found to be a class of salt-insensitive antibiotics potentially useful in the treatment of CF patients harboring multidrug resistant *P. aeruginosa.*

The last 15 years have witnessed the introduction of novel pharmacological interventions which may delay the inexorable decline phase of CF by promoting chloride efflux with uridine triphosphate (Knowles *et al.*, 1991), improving mucolysis with recombinant DNase (Shak *et al.*, 1990), neutralizing intraluminal proteolytic enzyme activity with aerosolized α1-anti-trypsin (McElvaney *et al.*, 1991), or recombinant secretory leukoprotease inhibitor (McElvaney *et al.*, 1992). Gene therapy could, in the foreseeable future, evolve as a therapeutic strategy directed towards arresting or circumventing the basic defect.

Antibiotic chemotherapy on the different Bcc species

The collection of 19 strains of Bcc examined in this study included examples of the first five genomovars described in the Bcc. Few studies have been performed on the susceptibility of these different genomovars. A study on antibiotic susceptibilities of Bcc strains by Aaron *et al.* (2000) looked at mainly *B. cenocepacia* strains so comparisons could not be made. As previously discussed, although all nine species comprising the Bcc are capable of airway infection in CF patients, genomovars I-V are the most commonly isolated from such patients, in particular, *B. cenocepacia* and *B. multivorans*, and also the most clinically relevant, in particular, *B. cenocepacia* (Mahenthiralingham., *et al.*, 2000a). *B. multivorans* are also commonly recovered from such patients. *B. vietnamiensis, B. stabilis* and *B. cepacia* represent minor fractions of CF lung infections (LiPuma *et al.*, 1999). It would be ideal to find an antibiotic combination which demonstrates synergy against all species of the Bcc, but it is important to confirm that such combinations have activity against *B. cenocepacia* isolates and *B. multivorans* in particular, due to there prevalence and significance within the CF community.

As Figure 4.7 illustrates, synergy was observed in 40 % of *B. cenocepacia* isolates with the antibiotic combinations of alafosfalin with ceftazidime, and ceftazidime with tobramycin in this study. Synergy was also observed in a number of *B. cenocepacia* isolates (30 %) when alafosfalin was in combination with meropenem. Among the ten *B. cenocepacia* strains were three ET12 clones. Synergysm with alafosfalin combined with meropenem was observed in two of these strains, and to alafosfalin with ceftazidime, and ceftazidime with tobramycin in the third strain. Synergy was observed

in some percentage of *B. multivorans* and *B. stabilis* strains with most antibiotic combinations tested apart from ciprofloxacin with alafosfalin, and tobramycin with alafosfalin. However, synergy was not demonstrated between these combinations in any Bcc strains. Synergy between alafosfalin and ceftazidime was observed against a number of *B. multivorans,* and in particular, *B. stabilis* and *B. vietnamiensis* strains. It could be that different virulence factors as yet unknown, present in particular strains play a role in their demonstrable resistance to antibiotics and antibiotic synergistic combinations adding to their virulence potential. The two *B. vietnamiensis* strains tested were not susceptible to many antibiotic combinations but synergy was demonstrated in both strains with the combinations of ceftazidime with alafosfalin, and meropenem with alafosfalin. Although the two strains of *B. cepacia* showed no susceptibility to any combinations tested, this is less of a concern as prevalence is low compared to the more pathogenic and transmissible *B. cenocepacia* for example. Due to the low numbers of each Bcc species tested, this interpretation can only be used as a guide. These observations on the whole add strength to ceftazidime being useful in combination with alafosfalin for CF Bcc infections. A large scale screening involving large numbers of each Bcc species, including the more newly described species, is warranted to see if antibiotic susceptibility does indeed differ between the species.

In conclusion, alafosfalin was most effective in combination with ceftazidime for treatment of Bcc strains, and in combination with tobramycin and ceftazidime as a triple combination for *P. aeruginosa*, although a greater percentage of Bcc strains were susceptible to tobramycin and ceftazidime without the addition of alafosfalin. It is likely that the synergistic effect observed between alafosfalin and ß-lactam antibiotics is due to a "multiblockade" cell wall synthesis phenomenon, as little synergy was observed

between alafosfalin and non-cell wall acting antibiotics. Due to the high levels of resistance of organisms such as Bcc and *P. aeruginosa* strains in CF patients lungs to commonly used anti-pseudomonal drugs, it is important that any potential alternative therapies are investigated. Novel molecules such as garenoxacin, gatifloxacin and DAPs should therefore be incorporated in future synergy studies, as well as compounds such as alafosfalin, which have been overlooked for treatment against such infections.

This study has highlighted the need for investigation into the susceptibilities of the different Bcc species to antibiotics currently used, and also to novel therapies which will undoubtedly be developed in the future.

CHAPTER 5

Final discussion and future research

Due to the complicated taxonomy of the Bcc (still undergoing review), identification of these organisms as a group, and even more so, of the individual species comprising the complex, remains challenging, particularly phenotypic identification. This thesis has covered three different aspects of the Bcc: genotypic and phenotypic identification, along with susceptibility testing.

Chapter 2 demonstrated that *B. cenocepacia* (III-A) was responsible for most Bcc infections in CF patients attending the Freeman Hospital Cardiopulmonary Transplantation Unit in Newcastle upon Tyne, followed by *B. multivorans*, and that the ET-12 clone was responsible for the majority of these *B. cenocepacia* III-A infections. This was not unexpected in comparison to previous studies carried out in other transplant units in the UK and US. The study also suggests that where pre and post transplant colonisation occurs in the lungs of CF patients, the same strain is most likely responsible for both colonisations. It is possible therefore that Bcc strains colonise the upper respiratory tract pre-transplantation in these individuals and then infect the new lungs post-transplantation. Environmental acquisition of the same strain is also a possibility. The microbanked organism collection at the Freeman Hospital offers an ever increasing collection of clinical Bcc isolates from respiratory samples taken from CF patients. This collection could lend itself to numerous studies on these organisms. Further research could therefore include, for example, continuous monitoring of Bcc species isolated from individual patients, including pre and post transplant isolates where available, to assess whether particular Bcc species replace one another, such as reported with *B. cenocepacia* and *B. multivorans* (Mahenthiralingam et al., 2001a), or colonise CF patients' lungs concomitantly.

The epidemiological study of Bcc strains isolated from such patient groups is extremely important, as this study has shown. Following the discovery that the ET-12 strain of *B. cenocepacia* III-A was associated with poor transplant survival, and was the most prevalent strain amongst *B. cenocepacia*-infected patients awaiting transplant, the Freeman Hospital Cardiopulmonary Transplantation Unit has altered its previously described peri-transplant management of patients infected with *B. cenocepacia* (De Soyza *et al.*, 2001). As a consequence this unit has now successfully transplanted two ET-12 infected patients with current survival times of up to one year. It remains to be elucidated if these measures will provide safer peri-transplant management strategies for patients infected with other *B. cenocepacia* strains.

Chapter 3 centred on the development of a phenotypic identification scheme for members of the Bcc. Current phenotypic techniques are somewhat limited in their ability to achieve differentiation between these species and improvement using enzyme substrates offers the possibility of a more rapid and accurate diagnosis. It is very interesting that following the screening for such a large number of enzymes within species of the Bcc, there was little discrimination observed between the species, in particular between *B. cepacia* and *B. cenocepacia*, (including both *recA* lineages III-A and III-B). Although no absolute differentiation of the Bcc species was found using detection of the enzymes sought in this project, the phenotypic heterogeneity within each species reported by numerous other authors was confirmed.

Following on from the extensive enzyme screening exercise, the possibility of applying such enzyme substrates to culture media for Bcc identification was explored. The ultimate goal would be to produce a chromogenic medium that could identify each

individual Bcc species. This would entail production of specific enzymes from each species that hydrolysed chromogenic enzyme substrates incorporated within the medium. Although such a medium has not yet been produced, studies such as those described in this thesis need to be followed up with further enzyme screening using novel substrates to enable the possibility of such a medium to be established. If, as this study suggests, enzyme substrates capable differentiating between all Bcc species are not available, a medium in which *B. cenocepacia*, and perhaps *B. multivorans* colonies produced a different colour to all the other Bcc species would also be desirable, as these are the most commonly isolated, and the most clinically significant Bcc species colonising CF patients. As the indoxyl substrates evaluated in the chromogenic strips were only tested using very small numbers of organisms, it would be of value to conduct further studies with a much larger sample of Bcc strains. Although not commonly isolated from CF patients, these studies should also include strains of Bcc genomovars VI-IX, such as those included in the updated version of the Bcc experimental strain panel published by Coenye *et al.* (2003), and also *B. ubonensis* if this is shown to be a tenth member of the Bcc. As Table 3.16 shows, a number of the substrates were hydrolysed by the majority of Bcc strains but by few *P. aeruginosa* strains. Although *P. aeruginosa* strains can be easily eliminated from culture media specific for Bcc, with addition of antibiotics for example, this perhaps offers opportunity for development of a medium which could isolate *P. aeruginosa* and Bcc on the same culture plate, with Bcc colonies distinguished from those of *P. aeruginosa* by their colour production.

Chapter 4 looked at the susceptibility of Bcc and *P. aeruginosa* strains to the phosphonopeptide alafosfalin in combination with a number of cell wall-acting

antibiotics. A number of these combinations were found to demonstrate synergy against a favourable percentage of the organisms tested, however, these percentages were no improvement on those reported in previous studies performed with other antibiotic combinations. Synergy studies such as these are extremely important for CF patient multi-resistant isolates such as Bcc strains and *P. aeruginosa.* Antibiotics, alternative to those commonly used in the treatment of such infections, should be constantly sought and evaluated in such synergy studies. These may include novel antimicrobial compounds, or, as in the case of alafosfalin, antibiotics used in the past for infections other than Bcc isolates. This study has also highlighted the lack of information available on the antibiotic susceptibility of the different species of the Bcc. Large scale susceptibility tests on the different Bcc species, in particular using frequently administered anti-pseudomonal drugs, is warranted.

In conclusion, with regards to the identification of Bcc strains, it would seem sensible to suggest that if laboratory facilities are unavailable for specialised testing, new isolates should be sent to referral laboratories capable of state of the art identification of the different Bcc species. However, thorough biochemical analysis may be used as a primary screen for the genomovar or species status or to confirm the identification of clinical isolates previously identified by molecular methods. Identification is more likely to be accurate if phenotyping is performed in combination with molecular testing such as that reported by Henry *et al.* (2001). A greater understanding of the health risks associated with infection by each genomovar or species can only be obtained if isolates are accurately identified.

REFERENCES

Aaron, S.D., Ferris, W., Henry, D.A., Speert, D.P., MacDonald, N.E. 2000. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. *American Journal of Respiratory Critical Care Medicine* **161**, 1206-1212

Agodi, A., Mahenthiralingam, E., Barchitta, M., Giannino, V., Sciacca, A., Stefani, S. 2001. *Burkholderia cepacia* complex infection in Italian patients with cystic fibrosis: prevalence, epidemiology and genomovar status. *Journal of Clinical Microbiology* **39**, 2891-2896

Ahmad, S., Ward, P.E. 1990. Role of aminopeptidase activity in the regulation of the pressor activity of circulating angiotensins. *Journal of Pharmacology and Experimental Therapeutics* **252**, 643-650

Al-Bakri, A.G., Gilbert, P., Allison, D.G. 2004. Immigration and emigration of *Burkholderia cepacia* and *Pseudomonas aeruginosa* between and within mixed biofilm communities. *Journal of Applied Microbiology* **96**, 455-463

Al-Thani, R. 1998. Interstrain comparison of some methicillin-resistant and methicillin-sensitive strains of *Staphylococcal aureus*. PhD thesis. University of Newcastle upon Tyne

Alkawash, M., Head, M., Alshami, I., Soothill, J.S. 1999. The effect of human lactoferrin on the MICs of doxycycline and rifampicin for *Burkholderia cepacia* and *Pseudomonas aeruginosa* strains. *Journal of Antimicrobial Chemotherapy* **44**, 385-387

Allen, J.G., Atherton, F.R., Hall, M.J., Hassall, C.H., Holmes, S.W., Lambert, R.W., Nisbet, L.J., Ringrose, P.S. 1978. Phosphonopeptides, a new class of synthetic antibacterial agents. *Nature* **272**, 56-58

Allen, J.G., Atherton, F.R., Hall, M.J., Hassall, C.H., Holmes, S.W., Lambert, R.W., Nisbet, L.J., Ringrose, P.S. 1979a. Phosphonopeptides as antibacterial agents: alafosfalin and related phosphonodipeptides. *Antimicrobial Agents and Chemotherapy* **15**, 684-695

Allen, J.G., Havas, L., Leicht, E., Lenox-Smith, I., Nisbet, L.J. 1979b. Phosphonopeptides as antibacterial agents: metabolism and pharmacokinetics of alafosfalin in animals and humans. *Antimicrobial Agents and Chemotherapy* **16**, 306-313

Allen, J.G., Lees, L.J. 1980. Pharmacokinetics of alafosfalin, alone and in combination with cephalexin,in humans. *Antimicrobial Agents and Chemotherapy* **17**, 973-979

Anderson, D.J., Kuhns, J.S., Vasil, M.L., Gerding, D.N., Janoff, E.N. 1991. DNA fingerprinting by Pulsed Field Gel Electrophoresis and Ribotyping to distinguish *Pseudomonas cepacia* isolates from a nosocomial outbreak. *Journal of Clinical Microbiology* **29**, 648-649

Andrews, J.M. 2001. BSAC standardized disc susceptibility testing method. *Journal of Antimicrobial Chemotherapy* **48**, 43-57

Andrews, J., Walker, R, King, A. 2002. Evaluation of media available for testing the susceptibility of *Pseudomonas aeruginosa* by BSAC methodology. *Journal of Antimicrobial Chemotherapy* **50**, 479-486

Anon. 1952. Respiratory tract involvement in cystic fibrosis of the pancreas (mucoviscidosis). *Journal of the American Medical Association* **150**, 1407.

Arbeit, R.D. 1995. Laboratory procedures for the epidemiological analysis of microorganisms. In: *Manual of Clinical Microbiology*, 6th edition. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H. (Eds.) p190-208. ASM Press, Washington D.C.

Aris, R.M., Routh, J.C., LiPuma, J.J., Heath, D.G., Gilligan, P.H. 2001. Lung transplantation for cystic fibrosis patients with *Burkholderia cepacia* complex. Survival linked to genomovar type. *American Journal of Respiratory Critical Care Medicine* **164**, 2102-2106

Arisawa, M., Ohshima, J., Ohsawa, E., Maruyama, H.B. 1982. *In vitro* potentiation of cephalosporins by alafosfalin against urinary tract bacteria. *Antimicrobial Agents and Chemotherapy* **21**, 706-710

Aronoff, S.C., Klinger, J.D. 1984. *In vitro* activities of aztreonam, piperacillin, and ticarcillin combined with amikacin against amikacin-resistant *P. aeruginosa* and *P. cepacia* isolates from children with cystic fibrosis. *Antimicrobial Agents and Chemotherapy* **25**, 279-280

Aronoff, S.C., Quinn, F.J., Stern, R.C. 1991. Longitudinal serum IgG response to *Pseudomonas cepacia* surface antigens in cystic fibrosis. *Pediatric Pulmonology* **11**, 289-293

Atherton, F.R., Hall, M.J., Hassall, C.H., Lambert, R.W., Lloyd, W.J., Ringrose, P.S. 1979a. Phosphonopeptides as Antibacterial agents: mechanism of action of alafosfalin. *Antimicrobial Agents and Chemotherapy* **15**, 696-705

Atherton, F.R., Hall, M.H., Hassall, C.H., Holmes, S.W., Lambert, R.W., Lloyd, W.J., Nisbet, L.J., Ringrose, P.S., Westmacott, D. 1981. Antibacterial properties of alafosfalin combined with cephalexin. *Antimicrobial Agents and Chemotherapy* **21**, 706-710

Balandreau, J., Viallard, V., Cournoyer, B., Coenye, T., Laevens, S., Vandamme, P. 2001. *Burkholderia cepacia* genomovar III is a common plant-associated bacterium. *Applied Environmental Microbiology* **67**, 982-985

Ballard, R.W., Palleroni N.J. 1970 Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia, P. marginator, P. allicola* and *P. carophylli. Journal of General Microbiology* **60**, 199-214

Banerjee, D., Stableforth, D. 2000. The treatment of respiratory pseudomonas infection in cystic fibrosis. What drug and which way? *Drugs* **60**, 1053-1064

Bascomb, S.A. 1987. Enzyme tests in bacterial identification. In: *Methods in Microbiology,* Vol 19. Colwell, R.R, Grigora, R. (Eds.) p 105-160. Academic Press, London

Bauernfeind, A., Schneider, I., Jungwirth, R., Roller, C. 1999. Discrimination of *B. multivorans* and *B. vietnamiensis* from *B. cepacia* genomovars I, III and IV. *Journal of Clinical Microbiology* **37**, 1335-1339

Beckman, W., Lessie, T.G. 1979. Response of *Pseudomonas cepacia* to ß-lactam antibiotics: utilization of penicillin G as the sole carbon source. *Journal of Bacteriology* **140**, 1126-1128

Berdal, B., P., Olsvik, O. 1983. Legionella extracellular protease activity on chromogenic peptides: a simplified procedure for biochemical enzyme identification. *Acta Pathologica et Microbiologica Scandinavia* **91**, 89-93

Berenbaum, M.C. 1978. A method for testing synergy with any number of agents. *Journal of Infectious Diseases* **127**, 122-130

Berenbaum, M.C. 1983. Correlations between methods for measurement of synergy. *Journal of Infectious Diseases* **142**, 476-478

Bevivino, A., Tabacchioni, S., Chiarini, L., Carusi, M.V., Del Gallo, M., Visca, P. 1994. Phenotypic comparison between rhizosphere and clinical isolates of *Burkholderia cepacia*. *Microbiology* **140**, 1069-1077

Bevivino, A., Dalmastri, C., Tabacchioni, S., Chiarini, L., Belli, M.L., Piana, S., Materazzo, A.,Vandamme, P. 2002. *Burkholderia complex* bacteria from clinical and environmental sources in Italy: genomovar status and distribution of traits related to virulence and transmissibility. *Journal of Clinical Microbiology* **40**, 846-851

Biddick, R., Spilker, T., Martin, A., LiPuma, J.J. 2003. Evidence of transmission of *Burkholderia cepacia, Burkholderia multivorans* and *Burkholderia dolosa* among persons with cystic fibrosis. *FEMS Microbiology Letters* **228**, 57-62

Bingen, E.D., Weber., M.O., Derelle, J., Brahimi, N., Lambert-Zechoovsky, N.Y., Vidailhet, M., Navarro, J., Elion, J. 1993. Arbitrarily primed polymerase chain reaction as a rapid method to differentiate crossed from independent *Pseudomonas cepacia* infections in cystic fibrosis patients. *Journal of Clinical Microbiology* **31**, 2589-2593

Bitton, G., Koopman, B., Jung, K. 1995. An assay for the enumeration of total coliforms and *Escherichia coli* in water and wastewater. *Water Environment Research* **67**, 906-909

Bonacorsi, S., Fitoussi, F., Lhopital, S., Bingen, E. 1999. Comparative in vitro activities of meropenem, imipenem, temocillin, piperacillin and ceftazidime in combination with tobramycin, rifampicin, or ciprofloxacin against *Burkholderia cepacia* isolates from patients with cystic fibrosis. *Antimicrobial Agents and Chemotherapy* **43**, 213-217

Bosso J.A., Saxon B.A., Matsen J.M. 1987. *In vitro* activity of aztreonam combined with tobramycin and gentamicin against clinical isolates of *P. aeruginosa* and *P. cepacia* from patients with cystic fibrosis. *Antimicrobial Agents and Chemotherapy* **31**, 1403-1405

Bosso, J.A., Saxon, B.A., Matsen, J.M. 1990. *In vitro* activities of combinations of aztreonam, ciprofloxacin, and ceftazidime against clinical isolates of *Pseudomonas aeruginosa* and *Pseudomonas cepacia* from patients with cystic fibrosis. *Antimicrobial Agents and Chemotherapy* **34**, 487-488

Brenner, K.P., Rankin, C.C., Roybal, Y.R., Stelma, G.N., Scarpino, P.V., Dufour, A.P. 1993. New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. *Applied and Environmental Microbiology* **59**, 3534-3544

Brisse, S., Verduin, C.M., Milatovic, D., Fluit, A., Verhoef, J., Laevens, S., Vandamme, P., Tummler, B., Verbrugh, H.A., van Belkum, A. 2000. Distinguishing species of the *Burkholderia cepacia* complex and *Burkholderia gladioli* by automated ribotyping. *Journal of Clinical Microbiology* **38**, 1876-1884

Brisse, S., Stefani, S., Verhoef, J., Van Belkum, A., Vandamme, P., Goessens, W. 2002. Comparative evaluation of the BD Phoenix and Vitek 2 automated instruments for identification of isolates of the *Burkholderia cepacia* complex. *Journal of Clinical Microbiology* **40**, 1743-1748

Brynes, P.J., Bevilacqua, P., Green, A. 1981. 6-aminoquinoline as a fluorogenic leaving group in peptide cleavage reactions: a new fluorogenic substrate for chymotrypsin. *Analytical Biochemistry* **116**, 408-413

Burbage, D.A., Sasser, M. 1982. A medium selective for *Pseudomonas cepacia. Phytopathology* **72**, 706

Bürger, H. 1967. Biochemical performance of non-proliferating microorganisms. II. Demonstration of glycoside hydrolases, phosphatases, esterases and lipases. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **202**, 97-109

Burkholder, W.H. 1950. Sour skin: a bacterial rot of onion bulbs. *Phytopathology* **40,** 15-23

Burnie, J.P., Al-Wardi, E.J., Williamson, P., Matthews, R.C., Webb, K., David, T. 1995. Defining potential targets for immunotherapy in *Burkholderia cepacia* infection. *FEMS Immunology and Medical Microbiology* **10**, 157-164

Burns, M.W., May, J.R. 1968. Bacterial precipitins in serum of patients with cystic fibrosis. *Lancet* **1**, 270-272

Burns, J., Wadsworth, C., Barry, J. 1989. Nucleotide sequence analysis of a gene from *Burkholderia (Pseudomonas) cepacia* encoding an outer membrane lipoprotein involved in multiple antibiotic resistance. *Antimicrobial Agents and Chemotherapy* **33**, 1247-1251

Burns, J.L. 1992. Characterisation of invasion of epithelial cells by *Pseudomonas cepacia*. *Pediatric Pulmonology* **8**, 201

Burns, J.L., Saiman, L., Whittier, S., Larone, D., Krzewinski, J., Zhenling, L., Marshall, S.A., Jones, R.N. 2000. Comparison of agar diffusion methodologies for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Journal of Clinical Microbiology* **38**, 1818-1822

Butler, S.L., Doherty, C.J. 1995, *Burkholderia cepacia* and cystic fibrosis: do natural environments present a potential hazard. *Journal of Clinical Microbiology* **33,** 1001-1004

Bye, M.R., Ewig, J., Quitell, L.M. 1994. Cystic Fibrosis. *Lung* **172**, 251-270

Campbell, P.W., Phillips, J.A., Heidecker, G.J., Krishnamani, M.R., Zahorchak, R., Stull, T.L. 1995. Detection of *Pseudomonas (Burkholderia) cepacia* using PCR. *Pediatric Pulmonology* **20**, 44-49

Cantin, A., Woods, D. 1993. Protection by antibiotics against myeloperoxidase-dependent cytotoxicity to lung epithelial cells in vitro. *Journal of Clinical Investigation* **91**, 38-45

Caparro, C., Maurer, J., Gutierrez, C., Krajden, M., Chan, C., Winton, T., Keshavjee, S., Scavuzzo, M., Tullis, E., Hutcheon, M., Kesten, S. 2001. Infection with Burkholderia cepcia in cystic fibrosis: outcome following lung transplantation. *American Journal of Respiratory Critical Care Medicine* **163**, 43-48

Carle, G.F., Olsen, M.V. 1984. Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. *Nucleic Acids Research* **12**, 5647-5664

Carson, L.R., Tablan, O.C., Cusick, L.B., Jarvis, W.R., Favero, M.S., Bland, L.A. 1988. Comparative evaluation of selective media for isolation of *P. cepacia* from cystic fibrosis patients and environmental sources. *Journal of Clinical Microbiology* **26**, 2096-3000

Castillo, M.J., Nakajima, K., Zimmerman, M., Powers, J.C. 1979. Sensitive substrates for human leukocyte and porcine pancreatic elastase: a study of the merits of various chromophoric and fluorogenic leaving groups in assays for serine proteases. *Analytical Biochemistry* **99**, 53-64

Cerantola, S., Marty, N., Montrozier, H. 1996. Structural studies of the acidic exopolysaccharide produced by a mucoid strain of *Burkholderia cepacia*, isolated from cystic fibrosis. *Carbohydrate Research* **285**, 59-67

Cerny, G. 1976. Methods for the distinction of Gram-negative from Gram-positive bacteria. *European Journal of Applied Microbiology* **32**, 223-225

Cerny, G. 1978. Studies on the aminopeptidase test for the distinction of Gram-negative from Gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology* **5**, 113-122

Chaparro, C., Maurer, J., Gutierrez, C., Krajden, M., Chan, C., Winton, T., Keshavjee, S., Scavuzzo, M., Tullis, E., Hutcheon, M., Kesten, S. 2001. Infection with *Burkholderia cepacia* in cystic fibrosis: outcome following lung transplantation. *American Journal of Respiratory Critical Care Medicine* **163**, 43-48.

Chen, J.S., Witzmann, K., Spilker, T., Fink, R., LiPuma, J.J. 2001. Endemicity and intercity spread of *Burkholderia cepacia* genomovar III in cystic fibrosis. *Journal of Pediatrics* **139**, 643-649

Cheng, H.P., Lessie, T.G. 1994. Multiple replicons constituting the genome of *Pseudomonas cepacia* 17616. *Journal of Bacteriology* **176**, 4034-4042

Chiu, C.H., Wong, S., Hancock, R.E., Speert, D.P. 2001. Adherence of *Burkholderia cepacia* to respiratory tract epithelial cells and inhibition with dextrans. *Microbiology* **147**, 2651-2658

Christenson, J.C., Welch, D.F., Mukwaya, G., Muszynski, M.J., Weaver, R.E., Brenner, D.J. 1989. Recovery of *Pseudomonas gladioli* from respiratory tract specimens of patients with cystic fibrosis. *Journal of Clinical Microbiology* **27**, 270-273.

Cimolai, N., Trombley, C. 1995. Insertional sequence primers for *Bordetella pertussis* diagnostic polymerase chain reaction differentiate strains of *Pseudomonas cepacia. Journal of Infectious Diseases* **172**, 293-295

Clode, F.E., Kaufman, M.E., Malnick, H., Pitt, T.L. 2000. Distribution of genes encoding putative transmissibility factors among epidemic and nonepidemic strains of *Burkholderia cepacia* from cystic fibrosis patients in the United Kingdom. *Journal of Clinical Microbiology* **38**, 1763-1766

Coburn, J.T., Lytle, F.E., Huber. 1986. Enhanced instrumental sensitivity and selectivity for aminopeptidase profiling. *Analytical Biochemistry* **154**, 305-310

Codd, A.A., Richardson, I.R., Andrews, N. 1998. Lenticules for the control of quantitative methods in food microbiology. *Journal of Applied Microbiology* **85**, 913-917

Coenye, T., Schouls, L.M., Govan, J.R.W., Kersters, K., Vandamme, P. 1999. Identification of Burkholderia species and genomovars from cystic fibrosis patients by AFLP fingerprinting. *International Journal of Systematic Bacteriology* **49**, 1657-1666

Coenye, T., Falsen, E., Hoste, B., Ohlen, M., Goris, J., Govan, J.R., Gillis, M., Vandamme, P. 2000. Description of *Pandoraea* gen. nov. with *Pandoraea apista* sp. nov., *Pandoraea pulmonicola* sp. nov., *Pandoraea pnomenusa* sp. nov., *Pandoraea sputorum* sp. nov. and *Pandoraea norimbergensis* comb. nov. *International Journal of Systematic and Evolutionary Microbiology* **50**, 887-899

Coenye, T., Vandamme, P., Govan, J.R.W., LiPuma, J.J. 2001a. Taxonomy and identification of the *B. cepacia* complex. *Journal of Clinical Microbiology* **39**, 3427-3436

Coenye, T., LiPuma, J.J., Henry, D., Hoste, B., Vandemeulebroecke, K., Gillis, M., Speert, D.P., Vandamme, P. 2001b. *Burkholderia cepacia* genomovar VI, a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients. *International Journal of Systematic and Evolutionary Microbiology* **51**, 271-279

Coenye, T., Mahenthiralingam, E., Henry, D., LiPuma, J.J., Hoste, B., Vandemeulebroecke, K., Gillis, M., Speert, D.P., Vandamme, P. 2001c. *Burkholderia ambifaria* sp. nov., a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. *International Journal of Systematic and Evolutionary Microbiology* **51**, 1481-1490

Coenye, T., LiPuma, J.J. 2002. Multilocus restriction typing, a novel tool for studying global epidemiology of *Burkholderia cepacia* complex infection in cystic fibrosis. *Journal of Infectious Diseases* **185**, 1454-1462

Coenye, T., Spilker, T., Martin, A., LiPuma, J.J. 2002. Comparative assessment of genotyping methods for epidemiologic study of *Burkholderia cepacia* genomovar III. *Journal of Clinical Microbiology* **40**, 3300-3307

Coenye, T., LiPuma, J.J. 2003. Molecular epidemiology of *Burkholderia* species. *Frontiers in Bioscience* **8**, 55-67

Coenye, T., Vandamme, P. 2003. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environmental Microbiology* **5**, 719-729

Coenye, T., Vandamme, P., LiPuma, J.J., Govan, J.R., Mahenthiralingam, E. 2003. Updated version of the *Burkholderia cepacia* complex experimental strain panel. *Journal of Clinical Microbiology* **41**, 2797-2798

Conway, B.A., Venu, V., Speert, D.P. 2002. Biofilm formation and acyl homoserine lactone production in the *Burkholderia cepacia* complex. *Journal of Bacteriology* **184**, 5678-5685

Conway, S.P., Brownlee, K.G., Denton, M., Peckham, D.G. 2003. Antibiotic treatment of multidrug-resistant organisms in cystic fibrosis. *American Journal of Respiratory Medicine* **2**, 321-332

Corey, M., Farewell, V. 1996. Determinants of mortality from cystic fibrosis in Canada, 1970-1989. *American Journal of Epidemiology* **143,** 1007-1017

Cox, A.D., Wilkinson, S.G. 1991. Ionizing groups in lipopolysaccharides of *Pseudomonas cepacia* in relation to antibiotic resistance. *Molecular Microbiology* **5,** 641-646

D`Amato, R.F., Enriquez, L.A., Tomfohrde, K.M., Singerman, E. 1978. Rapid identification of *Neisseria gonorrhoea* and *Neisseria meningitidis* by using enzymatic profiles. *Journal of Clinical Microbiology* **7**, 77-81

Dahlén, G., Linde, A. 1973. Screening plate method for detection of bacterial ß-glucuronidase. *Applied Microbiology* **26**, 863-866

Dasen, S.E., LiPuma, J.J., Kostman, J.R., Stull, T.L. 1994. Characterisation of PCR ribotyping for *Burkholderia cepacia*. *Journal of Clinical Microbiology* **32**, 2422-2441

Davidson, D.G., Dorin, J.R., McLachlan, G., Ranaldi, V., Lamb, D., Doherty, C., Govan, J., Porteous, D.J. 1995. Lung disease in the cystic fibrosis mouse exposed to bacterial pathogens. *Nature Genetics* **9**, 351-357

Dawis, M.A., Isenberg, H.D., France, K.A., Jenkins, S.G. 2003. *In vitro* activity of gatifloxacin alone and in combination with cefepime, meropenem, piperacillin and gentamicin against multidrug-resistant organisms. *Journal of Antimicrobial Chemotherapy* **51**, 1203-1211.

De Boer, E. 1998. Update on media for isolation of Enterobacteriaceae from foods. *International Journal of Food Microbiology* **45**, 43-53

De Soyza, A., McDowell, A., Archer, L., Dark, J.H., Elborn, S.J., Mahenthiralingam, E., Gould, K., Corris, P.A. 2001. *Burkholderia cepacia* complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. *Lancet* **358**, 1780-1781.

De Soyza, A., Archer, L., Wardle, J., Parry, G., Dark, J.H., Gould, K., Corris, P.A. 2003. Pulmonary transplantation for cystic fibrosis: pre-transplant recipient characteristics in patients dying if peri-operative sepsis. *Journal of Heart and Lung Transplantation* **22**, 764-769

De Soyza, A., Corris, P.A. 2003. Lung transplantation and the Burkholderia cepacia complex. *Journal of Heart and Lung Transplantation* **22**, 954-958

Dealler, S.F., Abbott, M., Croughan, M.J., Hawkey, P.M. 1989. Identification of *Branhamella catarrhalis* in 2.5 minutes with an indoxyl butyrate strip test. *Journal of Clinical Microbiology* **25**, 1390-1391

Dealler, S.F. 1993. Chromogenic and fluorogenic indicators and substrates in diagnostic microbiology. *Reviews in Medical microbiology* **4**, 198-206

Delisle, G.J., Ley, A. 1989. Rapid detection of *Escherichia coli* in urine samples by a new chromogenic beta-glucuronidase assay. *Journal of Clinical Microbiology* **27**, 778-779

Den Hollander, J.G., Mouton, J.W., Verbrugh, H.A. 1998. Use of pharmacodynamic parameters to predict efficacy of combination therapy by using fractional inhibitory concentration kinetics. *Antimicrobial Agents and Chemotherapy* **42**, 744-748

Di Pentima, M.C., Mason, E.O., Kaplan, S.L. 1998. *In vitro* antibiotic synergy against *Flavobacterium meningosepticum*: implications for therapeutic options. *Clinical Infectious Diseases* **26**, 1169-1176

Diaz, F., Mosovich, L.L., Neter, E. 1970. Serogroups of *Pseudomonas aeruginosa* and the immune response of patients with cystic fibrosis. *Journal of Infectious Diseases* **121**, 269-274

Doggett, R.G., Harrison, G.M., Wallis, E.S. 1964. Comparison of some properties of *Pseudomans aeruginosa* isolated from infectious in persons with and without cystic fibrosis. *Journal of Bacteriology* **87**, 427-431

Domingue, G., Ellis, B., Dasgupta, M., Costerton, J.W. 1994. Testing antimicrobial susceptibilities of adherent bacteria by a method that incorporates guidelines of the National Committee for Clinical Laboratory Standards. *Journal of Clinical Microbiology* **32**, 2564-2568

Donlan, R. M. 2001. Biofilm formation: a clinically relevant microbiological process. *Clinical Infectious Diseases* **33**, 1387-1392

Doring, G., Conway, S.P., Heijerman, H.G.M., Hodson, M.E., Hoiby, N., Smyth, A. 2000. Antibiotic chemotherapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *European Respiratory Journal* **16**, 749-767

Drabick, J.A., Gracely, E.J. 1996. Survival of *Burkholderia cepacia* on environmental surfaces. *Journal of Hospital Infection* **32**, 267-276

Drvinek, P., Hrbackova, H., Cinek, O., Bartosova, J., Nyc, O., Nemec, A., Pohunek. 2002. Direct PCR detection of *Burkholderia cepacia* complex and identification of its genomovars by using sputum as source of DNA. *Journal of Clinical Microbiology* **40**, 3485-3488

Drumm, M., Pope, H.A., Cliff, W.H., Rommens, J.M., Marvin, S.A., Tsui, L.C., Collins, F.S., Frizzel, R.A., Wilson, J.M. 1990. Correction of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer. *Cell* **62**, 1227-1233

Duff, A.J.A. 2002. Psychological consequences of segregation resulting from chronic *Burkholderia cepacia* infection in adults with CF. *Thorax* **57**, 756-758

Dyer, D.L. 1970. *Microbiological Detection and Identification System.* U.S. patent number 3,551,295

Edwards, P.R., Ewing, W.H. 1986. *Edwards and Ewing's Identification of Enterobacteriaceae* 4th Edition. Elsevier Science. New York.

Egan, J.J., McNeil, K., Bookless, B. 1994. Post-transplantation survival of cystic fibrosis patients with *Pseudomonas cepacia. Lancet 344*, 552-553

Eisen, J.A. 1995. The *recA* protein as a model molecule for molecular systematic studies of bacteria: comparison of trees of *recA*s and 16S rRNAs from the same species. *Journal of Molecular Evolution* **41**, 1105-1123

Elion, G.B., Singer, S., Hitchings, G.H. 1954. Antagonist of nucleic acid derivatives: synergism in combinations of biochemically related antimetabolites. *Journal of Biological Chemistry* **208**, 477-488.

Ellis, S., Walker, B.S. 1942. The action of hippuricase on ring substituted derivatives of hippuric acid. *Journal of Biological Chemistry* **142**, 291-297

Ensor, E., Humphreys, H. 1996. Is *Burkholderia (Pseudomonas) cepacia* disseminated from cystic fibrosis patients during physiotherapy. *Journal of Hospital Infection* **32**, 9-15

Facklam, R.R. 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. *Applied Microbiology* **23**, 1131-1139

Facklam, R.R., Thacker, L.G., Fox, B., Enriquez, L. 1982. Presumptive identification of streptococci with a new test system. *Journal of Clinical Microbiology* **15**, 987-990

Facklam, R.R., Rhoden, D.L., Smith, P.B. 1984. Evaluation of the strep system for the identification of clinical isolates of *Streptococcus* spp. *Journal of Clinical Microbiology* **22**, 894-898

Feng, P.C.S., Hartman, P.A. 1982. Fluorogenic assays for the immediate confirmation of *Escherichia coli*. *Applied Environmental Microbiology* **43**, 1320-1329

Ferroni, A., Sermet-Gaudelus, I., Abachin, E., Quesnes, G., Lenoir, G., Berche, P., Gaillard, J.L. 2003. Phenotypic and genotypic characteristics of non fermenting atypical strains recovered from cystic fibrosis patients. *Pathologie Biologie* **51**, 405-411

Fink, J., Steer, J.H., Joyce, D.A., McWilliam, A.S., Stewart, G.A. 2003. Pro-inflammatory effects of *Burkholderia cepacia* on cystic fibrosis respiratory epithelium. *FEMS Immunology and Medical Microbiology* **38**, 273-282

Fisher, M.C., LiPuma, J.J., Dasen, S.E., Caputo, G.C., Mortensen, J.E., McGowan, K.L., Stull, T.L. 1993. Source of *Pseudomonas cepacia*: ribotyping of isolates from patients and from the environment. *Journal of Pediatrics* **123**, 745-747

Frangolias, D.D., Mahenthiralingam, E., Rae, S. 1999. *Burkholderia cepacia* in cystic fibrosis. Variable disease course. *American Journal of Respiratory Critical Care Medicine* **160**, 1572-1577

Friberger, P., Knos, M., Gustavsson, S, Aurell, L., Claeson, G. 1979. *Chromogenic peptide substrates*. p121-127, Churchill Livingston, Edinburgh

Freier TA, Hartman PA. 1987. Improved membrane filtration media for enumeration of total coliforms and *Escherichia coli* from sewage and surface waters. *Applied Environmental Microbiology* **53**, 1246-1250

Fujiwara, K., Tsuru, D. 1978. New chromogenic and fluorogenic substrates for pyrrolidonyl peptidase. *Journal of Biochemistry* **83**, 1145-1149

Fung-Tomc, J.C., Gradelski, E., Valera, L., Huczko, E., Bonner, D.P. 2002. Synergistic activity of the novel des-fluoro(6) quinolone, garenoxacin (BMS-284756), in combination with other antimicrobial agents against *Pseudomonas aeruginosa* and related species. *International Journal of Antimicrobial Agents* **20**, 57-60

Gardiner, K. and Patterson, D. 1989. Transverse alternating field electrophoresis and applications to mammalian genome mapping. *Electrophoresis* **10**, 296

Geers, T., Baker, N. 1987. The effect of sublethal levels of antibiotics on the pathogenicity of *Pseudomonas aeruginosa* for tracheal tissue. *Journal of Antimicrobial Chemotherapy* **19**, 569-578

Ghuysen, J.M., Strominger, J.L., Tipper, D.J. 1967. In: *Comprehensive Biochemistry,* 26A. Florkin, M., Stotz, E.H. (Eds.) Elsevier, Amsterdam.

Ghuysen, J.M., Shockman, G.D. 1973. Biosynthesis of peptidoglycan. In: *Bacterial membranes and walls.* Leive (Ed.) p37-130. Decker Inc., New York.

Giammanco, G., Pignato, S., Agodi, A., Toucas, M., d'Hauteville, H. 1982. Taxonomic Value of a chromogenic test for the detection of aminopeptidases in the genus S*higella. Annales de l'Institut Pasteur Microbiologie* **133**, 343-346

Gilligan, P.H., Gage, P.A. 1985. Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory specimens of patients with cystic fibrosis. *Journal of Clinical Microbiology* **22**, 5-8

Gilligan, P.H. 1991. Microbiology of airway disease in patients with cystic fibrosis. *Clinical Microbiology Reviews* **4**, 35-51

Gilligan, P.H. 1995. *Pseudomonas* and *Burkholderia*. In: *Manual of Clinical Microbiology*, 7th Edition. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., (Eds.) p509-519. ASM Press, Washington D.C.

Gilligan, P.H., Whittier, S. 1999. *Burkholderia, Stenotrophomonas, Ralstonia, Brevundomonas, Comamonas* and *Acidovorax*. In: *Manual of Clinical Microbiology*, 7th Edition. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., (Eds.) p526-538. ASM Press, Washington D.C.

Gillis, M., Van, T.V., Bardin, R., Goor, M., Hebber, P., Willems, A., Segers, P., Kersters, K., Heulin, T., Fernandez, M.P. 1995. Polyphasic taxonomy in the genus *Burkholderia* leading to an amended description of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for N_2-fixing isolates from rice in Vietnam. *International Journal of Systematic Bacteriology* **45**, 274-289

Godard, C., Plesiat, P., Michel-Briand, Y. 1993. Persistance of *Pseudomonas aeruginosa* strains in seven cystic fibrosis patients followed over 20 months. *European Journal of Medicine* **2**, 117-120

Godsey, J.H., Matteo, M.R., Shen, D., Tolman, G., Goheke, J.R. 1981. Rapid identification of Enterobacteriaceae with microbial enzyme activity profiles. *Journal of Clinical Microbiology* **13**, 483-490

Gould, I.M., Wilson, D., Milne, K., Paterson, A., Golder, D., Russell, D. 1991. Interaction of imipenem with erythromycin and tetracycline assessed by microdilution checkerboard techniques. *Antimicrobial Agents and Chemotherapy* **35**, 2407-2409

Govan, J.R.W., Harris, G. 1985. Typing of *Pseudomonas cepacia* by bacteriocin susceptibility and production. *Journal of Clinical Microbiology* **22**, 490-494

Govan, J.R.W., Brown, P.H., Maddison, J., Doherty, C., Nelson, C.J., Dodd, M., Greening, A.P., Webb, A.K. 1993. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *Lancet* **342**, 15-19

Govan, J.R.W., Nelson, J.W. 1993. Microbiology of cystic fibrosis lung infections: themes and issues. *Journal of the Royal Society of Medicine* **86**, 11-18

Govan, J.R.W., Deretic, V. 1996. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *FEMS Microbiology Reviews* **60** 539-574.

Govan, J.R., Hughes, J.E., Vandamme, P. 1996. *Burkholderia cepacia*: medical, taxonomic and ecological issues. *Journal of Medical Microbiology* **45**, 395-407

Govan, J.R. 2000. Infection Control in cystic fibrosis: methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex. *Journal of the Royal Society of Medicine* **93**, 40-45

Grange, J.M., Clark, K. 1977. Use of umbelliferone derivatives in the study of enzymatic activities of mycobacterium. *Journal of Clinical Pathology* **30**, 151-153

Grange, J.M., McIntye, G., 1979. Fluorogenic glycoside substrates and their use in the identification of some slow growing mycobacteria. *Journal of Applied Bacteriology* **47**, 285-288

Grimwood, K., To, M., Rabin, H., Woods, D. 1989. Subinhibitory antibiotics reduce *Pseudomonas aeruginosa* tissue injury in the rat lung model. *Journal of Antimicrobial Chemotherapy* **24**, 937-945

Grimwood, K., To, M., Rabin, H., Woods, D. 1989. Inhibition of *Pseudomonas aeruginosa* exoenzyme expression by subinhibitory antibiotic concentrations. *Antimicrobial Agents and Chemotherapy* **33**, 41-47

Guilbault, G.G., Kramer, D.N. 1965. Resofurin butyrate and indoxyl acetate as fluorogenic substrates for cholinesterase. *Analytical Chemistry* **37**, 120-123

Gupta, K.G., Bhatnagar, L., Jain, A.K. 1974. Quantitative correlation between pyrrolidonyl peptidase activity and coagulase of staphylococci. *Annales de l'Institut Pasteur Microbiologie* **125**, 413-417

Hagedorn, C., Gould, W.D., Bardinelli, T.R., Gustavson, D.R. (1987). A selective medium for enumeration and recovery of *Pseudomonas cepacia* biotypes from soil. *Applied Environmental Microbiology* **53**, 2265-2268

Hall, M.J., Middleton, R.F., Westmacott, D. 1983. The fractional inhibitory concentration (FIC) index as a measure of synergy. *Journal of Antimicrobial Chemotherapy* **11**, 427-433

Hamilton-Miller, J.M. 1999. New BSAC sensitivity testing guidelines. *Journal of Antimicrobial Chemotherapy* **44**, 850-851

Hardy, K.A., McGowan, K.L., Fisher, M.C., Schidlow, D.V. 1986. *Pseudomonas cepacia* in the hospital setting: lack of transmission between cystic fibrosis patients. *Journal of Pediatrics* **109,** 51

Haughland, R.P. 1996. *Handbook of Fluorescent Probes and Research Products,* 7th Edition. Gregory, J. (Ed.) Molecular Probes, USA

He, G.X., Kuroda, T., Mima, T., Morita, Y., Mizushima, T., Tsuchiya T. 2004. An H(+)-coupled multidrug efflux pump, PmpM, a member of the MATE family of transporters from *Pseudomonas aeruginosa. Journal of Bacteriology* **186**, 262-265

Heath, D.G., Hohneker, K., Carriker, C., Smith, K., Routh, J., LiPuma, J.J., Aris, R.M., Weber, D., Gilligan, P.H. 2002. Six-year molecular analysis of *Burkholderia cepacia* Complex isolates among cystic fibrosis patients at a referral centre for lung transplantation. *Journal of clinical microbiology* **40**, 1188-1193

Henry, D.A., Campbell, M.E., LiPuma, J.J., Speert, D.P. 1997. Identification of *B. cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. *Journal of Clinical Microbiology* **35**, 614-619.

Henry, D.A., Campbell, M.E., McGimpsey, C., Clarke, A., Louden, L., Burns, J.L., Roe, M.H., Vandamme, P., Speert, D. 1999. Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. *Journal of Clinical Microbiology* **37**, 1004-1007

Henry, D.A., Mahenthiralingam, Vandamme, P., Coenye, T., Speert, D.P. 2001. Phenotypic methods for determining genomovar status of the *Burkholderia cepacia* complex. *Journal of Clinical Microbiology* **39**, 1073-1078

Hodson, M.E., Gallagher, C.G., Govan, J.R. 2002. Randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. *The European Respiratory Journal* **20**, 658-664

Hoiby, N., Johansen, H.K., Moser, C., Song, Z.J., Ciofu, O., Kharazmi, A. 2001. *Pseudomonas aeruginosa* and the biofilm mode of growth. *Microbes and Infection* **3**, 1-13

Hoiby, N. 2002. New antimicrobials in the management of cystic fibrosis. *Journal of Antimicrobial Chemotherapy* **49**, 235-238

Holmes, A., Govan, J., Goldstein, R. 1998. Agricultural use of *Burkholderia (Pseudomonas) cepacia*: A threat to human health? *Emerging Infectious Diseases* **4**, 221-227

Holmes, B., Howard, B.J. 1994. Nonfermentative Gram-negative bacteria. In: *Clinical and Pathogenic Microbiology*, 2nd Edition. Howard B.J., Keiser J.F. (Eds.) p337-367. Mosby, St Louis

Honicky, R., Harden, D., Hsu, J., Howatt, W., Kurlandsky, L., Homnick, D., Stull, T., LiPuma, J., Spohn, W., McCoy, K., Wilmott, R., Kanga, J., Christenson, J., Crozier, D., Reisman, J., Wilson, W., Ecclestone, E., Keeley, K., FitzSimmons, S., Beall, R. 1993. *Pseudomonas cepacia* at summer camps for persons with cystic fibrosis. *Morbidity and Mortality Weekly Report* **42**, 456-459

Horrevorts, A.M. de Ridder, C.M., Poot, M.C., de Jonge, M.J., Degener, J.E., Dzoljic-Danilovic, G. 1987. Chequerboard titrations: the influence of serial dilutions of antibiotics on the fractional inhibitory concentration index and fractional bactericidal concentration index. *Journal of Antimicrobial Chemotherapy* **19**, 119-125

Hosenpud, J.D., Bennet, L.E., Keck, B.M., Fiol, B., Boucek, M.M., Novick, R.J. 1999. The registry of the International Society for Heart and Lung Transplantation: sixteenth official report. *Journal of Heart and Lung Transplantation* **18**, 466-471

Hugh, R., Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *Journal of Bacteriology* **66**, 24-26

Humphreys, H., Peckham, D. 1994. Airborne dissemination of *Burkholderia (Pseudomonas) cepacia* from adult patients with cystic fibrosis. *Thorax*, **49**, 1157-1159

Husain, S., Singh N. 2002. *Burkholderia cepacia* infection and lung transplantation. *Seminars in Respiratory Infections* **17**, 284-290

Hutchinson, G.R, Parker, S. 1996. Home-use nebulizers: a potential primary source of *Burkholderia cepacia* and other colistin resistant, Gram-negative bacteria in patients with cystic fibrosis. *Journal of Clinical Microbiology* **34**, 584-587

Hwang, M., Ederer, G.M. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *Journal of Clinical Microbiology* **1**, 114-115

Iacocca, V. F., Sibinga, M.S., Barbero. G.J. 1963. Respiratory tract bacteriology in cystic fibrosis. *American Journal of Disease in Childhood* **106**, 316-324

Ieven, M. and Goossens, H. 1997. Relevance of nucleic acid amplification techniques for diagnosis of respiratory tract infections in the clinical laboratory. *Clinical Microbiology Reviews* **10**, 242-256

Imanaka, H., Kousaka, M., Tamura, G., Arima, K. 1965. Studies on pyrrolnitrin, a new antibiotic. Taxonomic studies on a pyrrolnitrin-producing strain. *Journal of Antibiotics.* **18**, 205-206

Isenburg H.D., Alperstein P., France K. 1999. *In vitro* activity of ciprofloxacin, levofloxacin, and trovafloxacin, alone and in combination with beta-lactams, against clinical isolates of *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and *Burkholderia cepacia. Diagnostic Microbiology and Infectious Disease* **33**, 81-86

Isles, A., Mclusky, I. 1984. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *Journal of Pediatrics* **104**, 206-210

Jaffe, A., Bush, A. 2001. Cystic Fibrosis: review of the decade. *Monaldi archives for chest disease* **56**, 240-247

James, A.J. 1994. Enzymes in taxonomy and diagnostic bacteriology. In: *Chemical Methods in Prokaryotic Systems*. O`Donnell, A.G. (Ed.) p471-492. John Wiley and Sons Ltd

James, A.L., Perry, J.D., Ford, M., Armstrong, L., Gould, F.K. 1996. Evaluation of cyclohexenoexculetin-ß-D-galactoside and 8-hydroxyquinoline-ß-D-galactoside as substrates for the detection of ß-galactosidase. *Applied and Environmental Microbiology* **62**, 3868-3870

James, A.L., Perry, J.D., Ford, M., Armstrong, L., Gould, F.K. 1997. Note: Cyclohexenesculetin-ß-glucoside: a new substrate for the detection of bacterial ß-D-glucosidase. *Journal of Applied Microbiology* **82**, 532-536

James, A.L., Perry, J.D., Chilvers, K., Robson, I.S., Armstrong, L., Orr, K.E. 2000. Alizarin-ß-D-galactosidase: a new substrate for the detection of bacterial ß-galactosidase. *Letters in Applied Microbiology* **30**, 336-340

Janda, W.M., Morello, J.A., Bohnhoff, M. 1984. Use of the API NeIdent system for identification of pathogenic *Neisseria spp.* and *Branhamella catarrhalis*. *Journal of Clinical Microbiology* **19**, 338-341

Jayaswal, R.K., Fernandez, M. 1993. Antagonism of *Pseudomonas cepacia* against phytopathogenic fungi. *Current Topics in Microbiology* **26**, 17-22

Johnson, W.M., Tyler, S.D., Rozee, K.R. 1994. Linkage analysis of geographical and clinical clusters in *Pseudomonas cepacia* infections by multilocus enzyme electrophoresis and ribotyping. *Journal of Clinical Microbiology* **32**, 924-930

Jonsson, V. 1970. Proposal for a new species *Pseudomonas kingii*, *International Journal of Systematic Bacteriology* **20**, 255-257

Joris, B., Galleni, M., Frere, J.M., Labia, R. 1994. Analysis of the *penA* gene of *Pseudomonas cepacia* 249. *Antimicrobial Agents Chemotherapy* **38**, 407-408

Kämpfer P., Dott W. 1988. Growth dependent enzymatic profiles of some Gram-negative Nonfermentative bacteria of clinical significance. *Zentralblatt fur Bakteriologie und Hygiene* **269**, 460-467

Kämpfer, P., Rauhoff, O., Dott., W. 1991. Glycosidase profiles of members of the family Enterobacteriaceae. *Journal of Clinical Microbiology* **29**, 2877-2879

Kämpfer, P. 1992. Differentiation of *Corynebacterium* spp., *Listeria* spp., and related organisms using fluorogenic substrates. *Journal of Clinical Microbiology* **30**, 1067-1071

Kämpfer, P., Tjernberg, I., Ursing, J. 1993. Numerical classification and identification of *Acinetobacter* genomic species. *Journal of Applied Bacteriology* **75**, 259-268

Karlin, S., Weinstock, G.M., Brendel, V. 1995. Bacterial classifications derived from *recA* protein sequence comparisons. *Journal of Bacteriology* **177**, 6881-6893

Karpati, F., Jonasson, J. 1996. Polymerase chain reaction for the detection of *Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Burkholderia cepacia* in sputum of patients with cystic fibrosis. *Molecular and Cellular Probes* **10**, 397-403

Keig, P.M., Ingham, E., Vandamme, P.A., Kerr, K.G. 2002. Differential invasion of respiratory epithelial cells by members of the *Burkholderia cepacia* complex. *Clinical Microbiology Infection* **8**, 47-49.

Kenna, D.T., Barcus, V.A., Langley, R.J., Vandamme, P., Govan, J.R. 2003. Lack of correlation between O-serotype, bacteriophage susceptibility and genomovar status in the *Burkholderia cepacia* complex. *FEMS Immunology and Medical Microbiology* **35**, 87-92

Kenwrick S, Patterson M, Speer A, Fischbeck K, Davies K. 1987. Molecular analysis of the Duchenne muscular dystrophy region using pulsed field gel electrophoresis. *Cell* **48**, 351-357

Kerr, J.R. 1993. In vitro activities of two drug combinations of ceftazidime, cefotaxime, cefuroxime, chloramphenicol, imipenem and temocillin against clinical isolates of *Pseudomonas cepacia* from patients with CF. *International Journal of Antimicrobial Agents* **3**, 205-209

Kilian, M., Bulow, P. 1976. Rapid diagnosis of Enterobacteriaceae: Detection of bacterial glycosidases. *Acta Pathologica et Microbiologica Scandinavia* **84**, 245-251

Kiska, D.L., Kerr, A., Jones, M.C., Caracciolo, J.A., Eskridge, B., Jordan, M., Miller, S., Hughes, D., King, N., Gilligan, P. 1996. Accuracy of four commercial systems for identification of *B. cepacia* and other Gram negative, non-fermenting bacilli recovered from patients with cystic fibrosis. *Journal of Clinical Microbiology* **34**, 886-891.

Kloosterziel, A.M., Wouters, J.T. 1976. Some properties of the lipase of Geotrichum candidum evaluated by a fluorimetric assay technique. *Journal of Applied Bacteriology* **40**, 293-299

Knowles, M.R., Clarke, L.L., Boucher, R.C. 1991. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *New England Journal of Medicine* **325**, 533-538

Koch, C., Hoiby, N. 1993. Pathogenesis of cystic fibrosis. *Lancet* **341**, 1065-1069

Koneman, E.W., Allen, S.D., Janda, W.M., Schrenenberger, P.C., Winn, W.C. (Eds.) 1992. Current rapid techniques and emerging technologies in the laboratories in the laboratory diagnosis of infectious diseases. *In Colour Atlas and Textbook of Diagnosis Microbiology,* 4th Edition. p1095-1115. J.B. Lippencott Company, Philadelphia

Kostman, J.R., Edlind, T.D., LiPuma, J.J., Stull, T.L. 1992. Molecular epidemiology of *Pseudomonas cepacia* determined by polymerase chain reaction ribotyping. *Journal of Clinical Microbiology* **30**, 2084-2087

Krogstad, D.J., Moellering, R.C. 1986. Antimicrobial Combinations. In *Antibiotics in Laboratory Medicine*, 3rd Edition. P537-595. Williams and Wilkins, Baltimore, MD

Kuehn, M., Lent, K., Haas, J., Hagenzieker, J., Cervin, M., Smith, A.L. 1992. Fimbriation of *Pseudomonas cepacia. Infection and Immunity* **60**, 2002-2007

Kumar, A., Wofford-McQueen, R., Gordon, R.C. 1989. Ciprofloxacin, imipenem and rifampicin: in-vitro synergy of two and three drug combinations against *Pseudomonas cepacia. Journal of Antimicrobial Chemotherapy* **23**, 831-835

Kumar, A., Dietrich, S., Schneider, W. 1997. Genetic relatedness *of Burkholderia (Pseudomonas) cepacia* isolates from five cystic fibrosis centrers in Michigan. *Respiratory Medicine* **91**, 485-492

Kuwada M, Katayama K. 1984. Differentiation of endopeptidases and aminopeptidases by high-performance liquid chromatography of reaction products from chromogenic peptide *p*-nitroanilines as substrates. *Analytical Biochemistry* **139**, 438-443.

Lacy, D.E., Smith, A.W., Stableforth, D.E., Smith, G., Weller, P.H., Brown, M.R.W. 1995. Serum IgG response to *Burkholderia cepacia* outer membrane antigens in cystic fibrosis: assessment of cross reactivity with *Pseudomonas aeruginosa. FEMS Immunology and Medical Microbiology* **10**, 253-262

Laffineur, K., Janssens, M., Charlier, J., Avesani, V., Wauters, G., Delmee, M. 2002. Biochemical and susceptibility tests useful for identification of non-fermenting Gram-negative rods. *American Society for Microbiology* **40**, 1085-1087

Lang, J.B., Aaron, S.D., Ferris, W., Hebert, P.C., MacDonald, N.E. 2000. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multi resistant strains of *Pseudomonas aeruginosa. American Journal of Respiratory Critical Care Medicine* **162**, 2241-2245

Laughon, B.E., Syed, S.A., Loesche, W.J. 1982. API-ZYM system for identification of *Bacteroides* spp., *Capnocytophaga* spp. and spirochaetes of oral origin. *Journal of Clinical Microbiology* **15**, 97-102

Lawrence, D. 2002. Clues to mechanism of *Pseudomonas* resistance in cystic fibrosis. *Lancet* **359**, 1410

Le Conte, P., Potel, G., Peltier, P., Horeau, D., Caillon, J., Juvin, M.E. 1993. Lung distribution and pharamokinetics of aerosolized tobramycin. *American Reviews of Respiratory Disease* **147**, 1279-1282

Le Minor, L., Ben Hamida, F. 1962. Avantages de la recherche de la ß-galactosidase sur celle de la fermentation du lactose en milieu complexe dans le diagnostic bacteriologique, en particulier des Enterobacteriaceae. *Annales de L`Institut Pasteur* **102**, 267-277

Lennon, E., DeCicco, B.T. 1991. Plasmids of *Pseudomonas cepacia* strains of diverse origins. *Applied and Environmental Microbiology* **57**, 2345-2350

Lewenza, S., Conway, B., Greenberg, E.P., Sokol, P.A. 1999. Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. *Journal of Bacteriology* **181,** 748-756.

Lewin, C., Doherty, C., Govan, J. 1993. In vitro activities of meropenem, PD 127391, PD 131628, ceftazidime, chloramphenicol, co-trimoxazole and ciprofloxacin against *Pseudomonas cepacia*. *Antimicrobial Agents and Chemotherapy* **37**, 123-125

Ley, A.N., Bowers, R.J., Wolfe, S. 1988. Indoxyl-ß-D-glucuronide, a novel chromogenic reagent for the specific detection and enumeration of *Escherichia coli* in environmental samples. *Canadian Journal of Microbiology* **34**, 690-693

Lightfoot N.F., Richardson I.R., Hartford J.P. 2001. The use of lenticules™ for the process control of enumeration techniques in food and environmental microbiology. *Journal of Applied Microbiology* **91**, 660-667

Liou, T.G., Adler, F.R., Fitzsimmons, S.C., Cahill, B.C., Hibbs, J.R., Marshall, B.C. 2001. Predictive 5-year survivorship model of cystic fibrosis. *American Journal of Epidemiology* **153**, 345-352.

LiPuma J.J., Mortensen, J.E., Dasen, S.E., Edlind, T.D., Schidlow, D.V., Burns, J.L., Stull, T.L. 1988. Ribotype analysis of *Pseudomonas cepacia* from cystic fibrosis treatment centres. *Journal of Paediatrics* **113**, 859-862

LiPuma, J.J., Dasen, S.E., Nielson, D.W., Stern, R.C., Stull, T.L. 1990. Person to person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. *Lancet* **336**, 1094-1096

LiPuma, J.J., Fisher, M.C., Dasen, S.C., Moertensen, J.E., Stull, T.L. 1991. Ribotype stability of serial pulmonary isolates of *Pseudomonas cepacia*. *Journal of Infectious Diseases* **164**, 133-136

Lipuma, J.J., Marks-Austin, K.A., Holsclaw, D.S., Jr, Winnie, G.B., Gilligan, P.H., Stull, T.L. 1994. Inapparent transmission of *Pseudomonas (Burkholderia) cepacia* among patients with cystic fibrosis. *Pediatric Infectious Disease Journal* **13**, 716-719

LiPuma, J.J. 1998a. *Burkholderia cepacia*, management issues and new insights. *Clinics in Chest Medicine* **19**, 473-485

LiPuma, J.J. 1998b. Burkholderia cepacia epidemiology and pathogenesis: implications for infection control. *Current opinion in pulmonary medicine* **4**, 337-341

LiPuma, J.J. 1999. *Burkholderia cepacia*, continuing the genomovar story. *Pediatric Pulmonary* **28**, 148

LiPuma, J.J., Dulaney, B.J., McMenamin, J.D., Whitby, P.W., Stull, T.L., Coenye, T., Vandamme, P. 1999. Development of rRNA-based PCR assays for the identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. *Journal of Clinical Microbiology* **37**, 3167-3170

LiPuma, J.J. Mahenthiralingam, E. 1999. Commercial use of *Burkholderia cepacia*. *Emerging Infectious Diseases* **5**, 305-306

LiPuma, J.J., Spilker, T, Gill, L.H., Campbell, P.W., Liu, L., Mahenthiralingam, E. 2001. Disproportionate distribution of *Burkholderia cepacia* complex speices and transmissibility markers in cystic fibrosis. *American Journal of Respiratory Critical Care Medicine* **164**, 92-96

LiPuma, J.J., Spilker, T., Coenye, T., Gonzalez, F. 2002. An epidemic *Burkholderia cepacia* complex strain identified in soil. *Lancet* **359,** 2002-2003

Lu, D.C., Chang, S.C., Chen, Y.C., Luh, K.T., Hsieh, W.C. 1997. *In vitro* activities of antimicrobial agents, alone and in combinations, against *Burkholderia cepacia* isolated from blood. *Diagnositc Microbiology and Infectious Disease* **28**, 187-191

MacFadden, J.F. (Ed.) 2000. *Biochemical tests for the identification of medical bacteria*, 3rd Edition. Lippencott Williams and Williams, Philadelphia

MacGowan, A. 2003. Synergy testing: is there a role in the treatment of cystic fibrosis patients? *International Congress and Symposium*, series number 254

Mackay, M.L., Milne, K., Gould, I.M. 2000. Comparison of methods for assessing synergic antibiotic interactions. *International Journal of Antimicrobial Agents* **15**, 125-129

Mahenthiralingam, E., Campbell, M.E., Henry, D.A., Speert, D.P. 1996. Epidemiology of *Burkholderia cepacia* infection in patients with cystic fibrosis: analysis by randomly amplified polymorphic DNA fingerprinting. *Journal of Clinical Microbiology* **34**, 2914-2920

Mahenthiralingam, E., Simpson, D.A., Speert, D.P. 1997. Identification and characterisation of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. *Journal of Clinical Microbiology* **35**, 808-816

Mahenthiralingam, E., Bischof, J., Byrne, S.K., Radomski, C., Davies, J.E., Av-Gay, Y. 2000a. DNA-based diagnostic approaches for the identification of *Burkholderia cepacia* complex bacterial pathogens, *Burkholderia vietnamiensis, Burkholderia multivorans, Burkholderia stabilis* and *Burkholderia cepacia* genomovars I and III. *Journal of Clinical Microbiology* **38**, 3165-3173

Mahenthiralingam, E., Coenye, T., Chung, J.W., Speert, D.P., Govan, J.R.W., Taylor, P., Vandamme, P. 2000b. Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* complex. *Journal of Clinical Microbiology* **38**, 910-913

Mahenthiralingam, E., Vandamme, P., Campbell, M.E. 2001. Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. *Clinical Infectious Diseases* **33**, 1469-1475

Mahenthiralingam, E., Vandamme, P., Campbell, M.E., Henry, D.A., Gravelle, A.M., Wong, L.T., Davidson, A.G., Wilcox, P.G., Nakielna, B., Speert, D.P. 2001a. Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. *Clinical Infectious Diseases* **33**, 1469-1475

Mahenthiralingam, E., Baldwin, A., Vandamme, P. 2002. *Burkholderia cepacia* complex infection in patients with cystic fibrosis. *Journal of Medical Microbiology* **51**, 533-538

Manafi, M., Kneifel, W. 1990. Rapid methods for differentiating Gram-positive from Gram-negative aerobic and facultative anaerobic bacteria. *Journal of Applied Bacteriology* **69**, 822-827

Manafi, M., Kneifel, W., Bascomb, S. 1991. Fluorogenic and chromogenic substrates used in bacterial diagnostics. *Microbiological reviews* **55**, 335-348

Manafi, M. 1996. Fluorogenic and chromogenic substrates in culture media and identication tests. *International Journal of Food Microbiology* **31**, 45-58

Manafi, M. 1998. Culture media containing fluorogenic and chromogenic substrates. *De Ware (n)-Chemicus* **28**, 12-17

Manafi, M. 2000. New developments in chromogenic and fluorogenic culture media. *International Journal of Food Microbiology* **60**, 205-218

Manno, G., Ugolotti, E., Belli, M.L., Fenu, M.L., Romano, L., Cruciani, M. 2003. Use of the E-test to assess synergy of antibiotic combinations against isolates of *B. cepacia*-complex from patients with cystic fibrosis. *European Journal of Clinical Microbiology and Infectious Diseases* **22**, 28-34

Marolda, C.L., Hauroder, B., John, M.A., Michel, R., Valvano, M.A. 1999. Intracellular survival and saprophytic growth of isolates from the *Burkholderia cepacia* complex in free-living amoebae. *Microbiology* **145**, 1509-1517

Martin, D.W., Mohr, C.D. 2000. Invasion and intracellular survival of *Burkholderia cepacia*. *Infection and Immunity* **68**, 24-29.

Martone, W.J., Tablan, O.C., Jarvis, W.R. 1987. The epidemiology of nosocomial epidemic *Pseudomonas cepacia* infections. *European Journal of Epidemiology* **3**, 222-232

Marshall, N., Andruszkiewicz, R., Gupta, S., Milewski, S., Payne, J.W. 2003. Structure-activity relationships for a series of peptidomimetic antimicrobial prodrugs containing glutamine analogues. *Journal of Antimicrobial Chemotherapy* **51**, 821-831

Maruyama, H.B., Ariwasa, M., Sawada, T. 1979. Alafosfalin, a new inhibitor of cell wall biosynthesis: *In vitro* activity against urinary isolates in Japan and potentiation with beta-lactams. *Antimicrobial Agents and Chemotherapy* **16**, 444-451

Mathee, K., Ciofu, O., Sterberg, C., Lindum, P.W., Campbell, J.I.A., Jenson, P. 1999. Mucoid conversion of *Pseudomonas aeruginosa* by hydrogen peroxide: a mechanism for virulence activation in the cystic fibrosis lung. *Microbiology* **145**, 1349-1357

McDowell, A., Mahenthiralingam, E., Moore, J.E., Dunbar, K.E., Webb, A.K., Dodd, M.E., Martin, S.L., Millar, B.C., Scott, C.J., Crowe, M., Elborn, J.S. 2001. PCR-based detection and identification of *Burkholderia cepacia* complex pathogens in sputum from cystic fibrosis patients. *Journal of Clinical Microbiology* **39**, 4247-4255

McElvaney, N.G., Hubbard, R.C., Birrer, P., Chernick, M.S., Caplan, D.B., Frank, M.M. 1991. Aerosol alpha1-trypsin treatment for cystic fibrosis. *Lancet* **337**, 392-394

McElvaney, N.G., Nakamura, H., Birrer, P., Hebert, C.A., Wong, W.L., Alphonso, M. 1992. Modulation of airway inflammation in cystic fibrosis: in vivo suppression of interleukin-8 levels on the respiratory epithelial surface by aerosolisation of recombinant secretory leukoprotease inhibitor. *Journal of Clinical Investigation* **90**, 1296-1301

McKenny, D., Brown, K.E., Allison, D.G. 1995. Influence of *Pseudomonas aeruginosa* exoproducts in virulence factor production in *Burkholderia cepacia*: evidence of interspecies communication. *Journal of Bacteriology* **177**, 6989-6992

McKevitt, A.I., Bajaksouzian, S., Klinger J.D., Woods, D.E. 1989. Purification and characterisation of an extracellular protease from *Pseudomonas cepacia. Infection and Immunity* **57**, 771-778

McLoughlin, T.J., Quinn, J.P., Bettermann, A., Bookland, R. 1992. *Pseudomonas cepacia* suppresion of sunflower wilt fungus and role of antifungal compounds in controlling the disease. *Applied and Environmental Microbiology* **58**, 1760-1763

McMenamin, J.D., Zaccone, T.M., Coenye, T., Vandamme, P., LiPuma, J.J. 2000. Misidentification of *Burkholderia cepacia* in U.S. cystic fibrosis treatment centres: an analysis of 1051 recent sputum isolates. *Chest* **117**, 1661-1665.

Millar-Jones, L., Paull, A., Saunders, Z., Goodchild, M.C. 1992. Transmission of *Pseudomonas cepacia* among cystic fibrosis patients. *Lancet* **340**, 491.

Miller, M.B. and Gilligan, P.H. 2003. Laboratory aspects of management of chronic pulmonary infections in patients with cystic fibrosis. *Journal of Clinical Microbiology* **41**, 4009-4015

Miller, D., Mahenthiralingam, E. 2003. Sequencing of the *Pseudomonas aeruginosa* and *Burkholderia cepacia* genomes and their applications in relation to cystic fibrosis. *Journal of the Royal Society of Medicine* **96**, 57-65

Miyamoto, T., Miwa, H., Hatono, S. 1990. Improved fluorogenic assay for detection of *Vibrio paphaemolyticus* in foods. *Applied and Environmental Microbiology* **56**, 1480-1484

Moore, J.E., Millar, B.C., Xu, J., Crowe, M., Redmond, A.O.B., Elborn, J.S. 2002a. Misidentification of a genomovar of *Burkholderia cepacia* by *recA* restriction fragment length polymorphism. *Journal of Clinical Pathology* **55**, 309-311

Moore, J.E., Reid, A., Millar, B.C., Jiru, X., McCaughan, J., Goldsmith, C.E., Collins, J., Murphy, P.G., Elborn, J.S. 2002b. *Pandoraea apista* isolated from a patient with cystic fibrosis: problems associated with laboratory identification. *British Journal of Biomedical Science* **59**, 164-165

Mortensen, J.E., Fisher, M.C., Lipuma, J.J. 1995, Recovery of *Pseudomonas cepacia* and other *Pseudomonas* species from the environment. *Infection Control and Hospital Epidemiology* **16**, 30-32

Muftic, M., 1967. Application of chromogenic substrates to the determination of peptidases in Mycobacteria. *Folia microbiologica* **12**, 500-507

Muhdi, K., Edenborough, F.P., Gumery, L. 1996. Outcome for patients colonized with *Burkholderia cepacia* in Birmingham adult cystic fibrosis clinic and the end of an epidemic. *Thorax* **51**, 374-377

Mulczyk, M., and Szweczuk, A. 1970. Pyrrolidonyl peptidase in bacteria: a new colorimetric test for differentiation of enterobacteriaceae. *Journal of General Microbiolo*. **61**, 9-13

Murgier, M., Pelissier, C., Lazdunski, A. 1976. Existence, Localisation and regulation of the biosynthesis of aminopeptidase in gram negative bacteria. *European Journal of Biochemistry* **65**, 517-520

Murray, P.R., Rosenthal, K.S., Kobayashi, G.S., Pfaller, M.A. (Eds.) 1998. Pseudomonas and related organisms. In: *Medical Microbiology* 3rd Edition. p258-264. Mosby-Year Book Inc

Narita, S., Eda, S., Yoshihara, E., Nakae, T. 2003. Linkage of the efflux-pump expression level with substrate extrusion rate in the MexAB-OprM efflux pump of *Pseudomonas aeruginosa*. *Biochemical and Biophysical Research Communications*. **308**, 922-926

Nelson, J.W., Butler, S.L., Brown, P.H., Greening, A.P., Govan, J.R.W. 1993. Serum IgG and sputum IgA antibody to core lipopolysaccharide antigen from *Pseudomonas cepacia* in patients with cystic fibrosis. *Journal of Medical Microbiology* **39**, 39-47

Nelson, J.W., Butler, S.L., Krieg, D., Govan, J.R.W. 1994. Virulence factors of *Burkholderia cepacia*. *FEMS Immunology and Medical Microbiology* **8**, 89-98

Neuman M. 1984. Recent developments in the field of phosphonic acid antibiotics. *Journal of Antimicrobial Chemotherapy* **14**, 309-311

O`Callaghan, E.M.O., Tanner, M.S., Boulnois, G.J. 1994. Development of a PCR probe test for identifying *Pseudomonas aeruginosa* and *Pseudomonas (Burkholderia) cepacia. Journal of Clinical Pathology* **47**, 222-226

Olive, D.M., Bean, P. 1999. Principles and applications of methods for DNA-based typing of microbial organisms. *Journal of Clinical Microbiology* **37**, 1661-1669

On, S.L.W & Holmes, B. 1992. Assessment of enzyme detection tests useful in identification of Campylobacter. *Journal of Clinical Microbiology* **30**, 746-749

Orbach, M.J., Vollrath, D., Davis, R.W., Yanofsky, C. 1988. *Molecular and Cell Biology* **8**, 1469

Owens, R.C, Banevicius, M.A., Nicolau. D.P., Nightingale, C.H., Quintiliani, R. 1997. *In vitro* synergystic activities of tobramycin and selected ß-lactams against 75 gram negative clinical isolates. *Antimicrobial Agents and Chemotherapy* **41**, 2586-2588

Palleroni, N.J., Holmes, B. 1981. *Pseudomonas cepacia* sp. nov., nom. rev. *International Journal of Systematic Bacteriology* **31**, 479-481

Palleroni, N.J. 1984. Genus I. *Pseudomonas* In: *Bergeys Manual of Systematic Bacteriology*, Vol. 1. Krieg, N.R., Holt, J.G. (Eds.) p141-199. Williams & Wilkins, Baltimore

Panosian, K.J., & Edberg, S. 1989. Rapid Identification of *Streptococcus bovis* by using combination constitutive enzyme substrate hydrolyses. *Journal of Clinical Microbiology* **27**, 1719-1722

Parke, J.L., Rand, R.E., Joy, A.E., King, E.B. 1991. Biological control of Pythium dampingoff and Aphanomyces root rot of peas by application of *Pseudomonas cepacia* or *P. fluorescens* to seed. *Plant Diseases* **75**, 987-992

Parr, T.R., Moore, R.A. 1987. Role of porins in intrinsic resistance of *Pseudomonas cepacia. Antimicrobial Agents and Chemotherapy* **31**, 121-123

Pegues, C.F., Pegues, D.A. 1996, *Burkholderia cepacia* respiratory tract acquisition: epidemiology and molecular characterisation of a large nosocomial outbreak. *Epidemiology and Infection* **116**, 309-317

Penketh, A.L., Wise, A., Mearns, M.B., Hodson, M.E., Batten, J.C. 1987. Cystic fibrosis in adolescents and adults. *Thorax* **42**, 526-532

Perez, J.L., Berrocal, C.I., Berrocal, L. 1986. Evaluation of a commercial ß-glucuronidase test for the rapid and economical identification of *Escherichia coli. Journal of Applied Bacteriology* **61**, 541-545

Perry, J.D., Archer, L., Jones, A.L., James, A.L., Ford., M., Gould, F.K. 1998. Detection of carboxypeptidases as taxonomic markers for Gram negative bacteria. *Letters in Applied Microbiology* **26**, 359-362

Perry, J.D., Taylor, M., Jones, A.L., Freeman, R., Gould, F.K. 1999. ABC medium, a new chromogenic agar for selective isolation of *Salmonella* spp. *Journal of Clinical Microbiology* **37**, 766-768

Peterson, E.H., Hsu, E.J. 1978. Rapid detection of selected Gram negative bacteria by aminopeptidase profiles. *Journal of Food Science* **43**, 1853-1856

Petrucca, A., Cipriana, P., Valenti, P., Santapaola, D., Cimmino, C., Scoarughi, G.L., Santino, I., Stefani, S., Sessa, R., Nicoletti, M. 2003. Molecular characterisation of *Burkholderia cepacia* isolates from cystic fibrosis (CF) patients in an Italian CF centre. *Research in Microbiology* **154,** 491-498

Pitt, T.L., Govan, J.R.W. 1993. *Pseudomonas cepacia* and cystic fibrosis. *PHLS Microbiology Digest* **10**, 69-72

Pitt, T.L., Kaufmann, M.E., Patel, P.S., Benge, L.G., Gaskin, S., Livermore, D.M. 1996. Type characterisation and antibiotic susceptibility of *Burkholderia cepacia* isolates from patients with cystic fibrosis in the United Kingdom and the Republic of Ireland. *Journal of Medical Microbiology* **44**, 203-210

Popovic-Uroic, T., Patton, C.M., Nicholson, M.A., Kiehlbauch, J.A. 1990. Evaluation of the indoxyl acetate hydrolysis test for rapid differentiation of *Campylobacter*, *Helicobacter*, and *Wolinella* species. *Journal of Clinical Microbiology* **28**, 2335-2339

Prince, A., Wood, M.S., Cacalano, G.S., Chin, N.S. 1988. Isolation and characterisation of a penicillinase from *Pseudomonas cepacia* 249. *Antimicrobial Agents and Chemotherapy* **32**, 838-843

Rabkin, C.S., Jarvis, W.R., Martone, W.J. 1987. Current status of *Pseudomonas cepacia* typing systems. *European Journal of Epidemiology* **3**, 343-346

Rabkin, C.S., Jarvis, W.R., Anderson, R.L., Govan, J., Klinger, J., LiPuma, J.J., Martone, W.J., Monteil, H., Richard, C., Shigeta, S., Sosa, A., Stull, T., Swenson, J., Woods, D. 1989. *Pseudomonas cepacia* typing systems: collaborative study to assess their potential in epidemiologic investigations. *Review of Infectious Diseases* **11**, 600-607

Ramsey, W.S., Nowlan, E.D., Simpson, L.B., Messing, R.A., Takeguchi, M.M. 1980. Applications of fluorophore-containing microbial growth media. *Applied and Environmental Microbiology* **39**, 372-375

Ramsey, B.W., Dorkin, H.L., Eisenberg, J.D., Gibson, R.L., Harwood, I.R., Kravitz, R.M. 1993. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *New England Journal of Medicine* **328**, 1740-1746

Rand, K.H., Houck, H.J., Brown, P., Bennett, D. 1993. Reproducibility of the microdilution checkerboard method for antibiotic synergy. *Antimicrobial Agents and Chemotherapy* **37**, 613-615

Regelmann, W., Elliott, G., Warwick, W.,Clawson, C. 1990. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than bronchodilators and chest physiotherapy alone. *American Review of Respiratory Diseases* **141**, 914-921

Riedel, K., Hentzer, M., Geisenberger, O., Huber, B., Steidle, A., Wu, H., Hoiby, N., Givskov, M., Molin, S., Eberl, L. 2001. N-acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* **147**, 3249-3262

Riordan, J.R., Rommens, J.M., Kerem, B.S., Alon, N., Rozmahel, R., Grzelczak, K., Zielenski, J., Lok, J., Plasic, S., Chou, J.L., Drumm, M.L., Ianuzzi, M.C., Collins, F.S., Tsui, L.C. (1989). Identification of the cystic fibrosis gene: cloning and characterisation of complementary DNA. *Science* **245**, 1066-1073

Röd, T.O., Haug, R.H., Midvedt, T. 1974. ß-glucuronidase in the streptococci groups B and D. *Acta Pathologica Microbiologica Scandinavia Section* **82**, 533-536

Romling, U., Fielder, B., Bosshammer, J., Grothues, D., Greipel, J., von der Hardt, H., Tummler, B. 1994. Epidemiology of chronic *Pseudomonas aeruginosa* infections in cystic fibrosis. *Journal of Infectious Diseases* **170**, 1616-1621

Rosenstein, B.J., Hall, D.E. 1980. Pneumonia and septicaemia due to *Pseudomonas cepacia* in a patient with cystic fibrosis. *The Johns Hopkins Medical Journal* **147**, 188-189

Ryan, G., Mukhopadhyay, S., Singh, M. 2000. Nebulised anti-pseudomonal antibiotics for cystic fibrosis. *Cochrane Database Systematic Reviews* **2**, CD001021

Sabate, J., Villanueva, A., Prieto, M.J. 1994. Isolation and characterisation of a mercury-resistant broad-host-range plasmid from *Pseudomonas cepacia. FEMS Microbiology Letters* **119**, 345-350

Saiman, L., Sadoff, J., Prince, A. 1989 cross-reactivity of *Pseudomonas aeruginosa* antipilin monoclonal antibodies with heterogeneous strains of *P. aeruginosa and P. cepacia. Infection and Immunity* **57**, 2764-2770

Saiman, L., Cacalano, G., Prince, A. 1990. *Pseudomonas cepacia* adherence to respiratory epithelial cells is enhanced by *Pseudomonas aeruginosa. Infection and Immunity* **58**, 2578-2584

Saiman, L., Mehar, F., Niu, W.W., Neu, H., Shaw, K.J., Miller, G., Prince, A. 1996. Antibiotic susceptibility of multiply resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, including candidates for transplantation. *Clinical Infectious Diseases* **23**, 532-537

Saiman, L. 1998. CF referral centre for susceptibility and synergy studies of resistant organisms, Columbia University, New York

Saiman L, Chen Y, Tabibi S, San Gabriel P, Zhou J, Liu Z, Lai L, Whittier S. 2001. Identification and antimicrobial susceptibility of Alcaligenes xylosoxidans isolated from patients with cystic fibrosis. *Journal of Clinical Microbiology* **39**, 3942-3945

Sajjan, U.S., Corey, M., Karmali, M.A., Forstner, J.F. 1992. Binding of *Pseudomonas cepacia* to normal human intestinal mucin and respiratory mucin from patients with cystic fibrosis. *Journal of Clinical Investigation* **89**, 648-656

Sajjan, U.S., Forstner, J.F. 1993. Role of a 22-kilodalton pilin protein in binding of *Pseudomonas cepacia* to buccal epithelial cells. *Infection and Immunity* **61**, 3157-3163

Sajjan, U.S., Sun, L., Goldstein, R., Forstner, J.F. 1995. Cable (cbl) type II pili of cystic fibrosis-associated *Burkholderia (Pseudomonas) cepacia*: nucleotide sequence of cblA major subunit pilin gene and novel morphology of the assembled appendage fibers. *Journal of Bacteriology* **177**, 1030-1038

Sanderink, G., Artur, Y., Schiele, F., Gueguen, R., Siest, G. 1988. Alanine aminopeptidase in serum: biological variations and reference limits. *Clinical Chemistry* **34**, 1422-1426

Schwab, U., Leigh, M., Ribeiro, C., Yankaskas, J., Burns, K., Gilligan, P., Sokol, P., Boucher, R. 2002. Patterns of epithelial cell invasion by different species of the *Burkholderia cepacia* complex in well-differentiated human airway epithelium. *Infection and Immunity* **70**, 4547-4555

Schwartz, D.C., Saffran, W., Welsh, J., Haas, R., Goldenberg, M., Cantor, C.R. 1982. Cold Spring Harbor Symposia on Quantitative biology **47**, 189

Schwartz, D.C., Cantor, C.R. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37**, 67-75

Segonds, C., Bingen, E., Couetdic, G., Mathy, S., Brahimy, N., Marty, N., Plesiat, P., Michel-Briand, Y., Chabanon, G. 1997. Genotypic analysis of *Burkholderia cepacia* isolates from 13 French cystic fibrosis centres. *Journal of Clinical Microbiology* **35**, 2055-2060

Segonds, C., Heulin, T., Marty, N., Chabanon, G. 1999. Differentiation of *Burkholderia* species by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene and application to cystic fibrosis isolates. *Journal of Clinical Microbiology* **37**, 2201-2208

Sekiya, H., Mima, T., Morita, Y., Kuroda, T., Mizushima, T., Tsuchiya, T. 2003. Functional cloning and characterization of a multidrug efflux pump, mexHI-opmD, from a *Pseudomonas aeruginosa* mutant. *Antimicrobial Agents and Chemotherapy* **47**, 2990-2992.

Shadix, L.C., Rice, E.W. 1991. Evaluation of ß-glucuronidase assay for the detection of Escherichia coli from environmental waters. *Canadian Journal of Microbiology* **37**, 908-911

Shak, S., Capon, H., Masters, S.A., Baker, C.L. 1990. Recombinant DNase I reduces the viscocity of cystic fibrosis sputum. *Proceedings of the National Academy of Sciences* **87**, 9188-9192

Shaw, D.P., Poxton, I.R., Govan, J.R.W. 1995. Biological activity of *Burkholderia (Pseudomonas) cepacia* lipopolysaccharide. *FEMS Immunology and Medical Microbiology* **11**, 99-106

Shawar, R.M., MacLeod, D.L., Garber, R.L., Burns, J.L., Stapp, J.R., Clausen, C.R.,Tanaka, S.K. 1999. Activities of tobramycin and six other antibiotics against *Pseudomonas aeruginosa* isolates from sputum of patients with cystic fibrosis. *Antimicrobial Agents and Chemotherapy* **43**, 2877-2880

Schwab, U., Gilligan, P., Jaynes, J., Henke, D. 1999. *In vitro* activities of designed antimicrobial peptides against multidrug-resistant cystic fibrosis pathogens. *Antimicrobial Agents and Chemotherapy* **43**, 1435-1440

Shelly, D.B., Spilker, T., Gracely, E. J., Coenye, T., Vandamme, P., LiPuma, J.J. 2000. Utility of commercial systems for identification of *Burkholderia cepacia* complex from cystic fibrosis sputum culture. *Journal of Clinical Microbiology* **38**, 3112-3115.

Shreve, M.R., Butler, S., Kaplowitz, H.J., Rabin, H.R., Stokes, D., Light, M., Regelmann, W.E. 1999. Impact of microbiology practice on cumulative prevalence of respiratory tract bacteria in patients with cystic fibrosis. Risk factors and outcomes. *Chest* **91**, 527-532

Skully, M.F., Kakkar, V.V. 1979. *Chromogenic peptide substrates*, Churchill Livingston, Edinburgh

Slots, J. 1981. Enzymatic characterisation of some oral and some non-oral Gram-negative bacteria with the API-ZYM system. *Journal of Clinical Microbiology* **14**, 288-294

Smith, C.L., Econome, J.G., Schutt, A., Klco, S., Cantor, C.R. 1987. *Science* **236**, 1448

Smith, A., Doershuk, C., Goldmann, D., Gore, E., Hilman, B. 1999. Comparison of a beta-lactam alone versus beta-lactam and an aminoglycoside for pulmonary exacerbation in cystic fibrosis. *Journal of Pediatrics* **134**, 413-421

Smith, D.L., Gumery, L.B., Smith, E.G., Stableforth, D.E., Kaufmann, M.E., Pitt, T.L. 1993. Epidemic of *Pseudomonas cepacia* in an adult cystic fibrosis unit: evidence of person-to-person spread. *Journal of Clinical Microbiology* **31**, 3017-3022.

Snell, J.J.S., Hill, L.R., Lapage, S.P. Curtis, M.A. 1972. Identification of *Pseudomonas cepacia* Burkholder and its synonymy with *Pseudomonas kingii* Jonsson. *International Journal of Systematic Bacteriology* **22**, 127-138

Snell, G.I., de Hoyos, A., Krajden, M., Winton, T., Maurer, J.R. 1993. *Pseudomonas cepacia* in lung transplant recipients with cystic fibrosis. *Chest* **103**, 466-471

Soper T.S., Manning J.M. 1976. Synergy in the antimicrobial action of penicillin and ß-chloro-D-alanine *in vitro*. *Antimicrobial Agents and Chemotherapy* **9**, 347-349

Speeleveld, E., Fossepre, J.M., Gordts, B., van Landuyt, H.W. 1994. Comparison of three methods, tributyrine, 4-methylumbelliferyl butyrate, and indoxyl acetate, for rapid identification of Moraxella catarrhalis. *Journal of Clinical Microbiology* **32**, 1362-1363

Speert, D., Henry, D., Vandamme, P., Corey, M., Mahenthiralingam, E. 2002. Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. *Emerging Infectious Diseases* **8**, 181-187

Spratt, B.G., Pardee, A.B. 1975. Penicillin-binding proteins and cell shape in *E. coli*. *Nature* **254**, 516-517

Stanier, R.Y., Palleroni, N.J., Doudoroff, M. 1966. The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159-271

Stead, R.J., Hodson, M.E., Batten, J.C. 1987. Inhaled ceftazidime compared with gentamicin and cabenicillin in older patients infected with *Pseudomonas aeruginosa*. *British journal of diseases of the chest* **81**, 272-278

Steinbach, S., Sun, L., Jiang, R.Z. 1994. Transmissibility of *Pseudomonas cepacia* infection in clinic patients and lung-transplant recipients with cystic fibrosis. *New England Journal of Medicine* **331**, 981-987

Stewart, P.S., Costerton, J.W. 2001. Antibiotic resistance of bacteria in biofilms. *Lancet* **358**, 135-138

Stirling CJ, Colloms SD, Collins JF, Szatmari G, Sherratt DJ. 1989. *xerB*, an Escherichia coli gene required for plasmid ColE1 site-specific recombination, is identical to *pepA*, encoding aminopeptidase A, a protein with substantial similarity to bovine lens leucine aminopeptidase. *The EMBO journal* **8**, 1623-1627

Stover, B.G., Drake, D.R., Montie, T.C. 1983. Virulence of different *Pseudomonas* species in a burned mouse model: tissue colonisation by P*seudomonas cepacia. Infection and Immunity* **41**, 1099-1104

Strominger, J.L. 1962. In: *The Bacteria.* Gunsalus, I.C., Stanier, R.Y. (Eds.) p413. Academic Press, London

Stull, T.L., LiPuma, J.J., Edlind, T.D. 1988. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. *Journal of Infectious Disease* **157**, 280-286

Sun, L., Jiang, R.Z., Steinbach, S. 1995. The emergence of a highly transmissible lineage of cbl+ *B. cepacia* causing CF centre epidemics in N. America and Britain. *Nature Medicine* **1**, 661-666

Svendsen, L., Blomback, B., Blomback, M., Ohlson, P.I. 1972. The assay of antithrombin using a synthetic chromogenic substrate for thrombin. *Thrombosis Res* **5**, 621-632.

Tabacchioni, S., Visca, P., Chiarini, L., Bevivino, A., Di Serio, C., Fancelli, S., Fani, R. 1995. Molecular characterisation of rhizosphere and clinical isolates of *Burkholderia cepacia. Research in Microbiology* **146**, 531-542

Tablan, O.C., Chorba, T.L., Schidlow, D.V., White, J.W., Hardy, K.A., Gilligan, P.H., Morgan, W.M., Carson, L.A., Martone, W.J., Jason, J.M., Jarvis, W.R. 1985. *Pseudomonas cepacia* colonisation in patietns with cystic fibrosis: risk factors and clinical outcome. *Journal of Pediatrics* **107**, 382-387

Tablan, O.C., Carson, L.A., Cusick, L.B., Bland, L.A., Martone, W.J., Jarvis, W.R. 1987. Laboratory proficiency test results on use of selective media for isolating *Pseudomonas cepacia* from simulated sputum specimens of patients with cystic fibrosis. *Journal of Clinical Microbiology* **25**, 485-487

Taylor, A., Brown, M.J., Daims, M.A., Cohen, J. 1983. Localization of leucine aminopeptidase in normal hog lens by immunofluorescence and activity assays. *Investigative ophthalmology & visual science* **24**, 1172-1180.

Taylor, A. 1993a. Aminopeptidases: towards a mechanism of action. *Trends in biochemical sciences* **18**, 167-171

Taylor, A. 1993b. Aminopeptidases: structure and function. *The FASEB Journal* **7**, 290-298

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* **33**, 2233-2239

Thomasson, M.J., Demko, C.A. 1985. *Pseudomonas cepacia* colonisation among patients with cystic fibrosis. *American Reviews of Respiratory Disease* **131**, 791-796

Touw, D.J., Brimicombe, RW., Hodson, M.E. 1995. Inhalation of antibiotics in cystic fibrosis. *European Respiratory Journal* **8**, 1594-1604

Trepanier, S., Prince, A., Huletsky, A. 1997. Characterization of the *penA* and *penR* genes of *Burkholderia cepacia* 249 which encode the chromosomal class A penicillinase and its LysR-type transcriptional regulator. *Antimicrobial Agents and Chemotherapy* **41**, 2399-2405.

Trepeta, R.W., Edberg, S.C. 1984. Methylumbelliferyl-beta-D-glucuronide-based medium for rapid isolation and identification of *Escherichia coli. Journal of Clinical Microbiology* **19**, 172-174.

Trepeta, R.W., Edberg, S.C. 1987. Esculinase (ß-glucosidase) for the rapid estimation of activity in bacteria utilising a hydrolysable substrate, p-nitrophenyl-ß-D-glucopyranoside. *Antonie van Leeuwenhoek* **53**, 273-277

Tummler, B., Kiewitz, C. 1999. Cystic fibrosis: an inherited susceptibility to bacterial respiratory infections. *Molecular Medicine Today* **5**, 351-358

Tunney, M.M., Scott, E.M. 2004. Use of breakpoint combination sensitivity testing as a simple and convenient method to evaluate the combined effects of ceftazidime and tobramycin on *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex isolates in vitro. *Journal of Microbiological Methods* **57**, 107-114

Tyler, S.D., Rozee, K.R., Johnson, W.M. 1996. Identification of IS1356, a new insertion sequence, and its association with IS402 in epidemic strains of *Burkholderia cepacia* infecting cystic fibrosis patients. *Journal of Clinical Microbiology* **34**, 1610-1616

UK Cystic Fibrosis Trust Infection Control Group. 1999. *Burkholderia cepacia.* Bromley, Kent: The CF Trust.

UK Cystic Fibrosis Trust Antibiotic Group. 2000. Report: Antibiotic treatment for cystic fibrosis, p46

Valerius, N.H., Koch, C., Hoiby, N. 1991. Prevention of chronic *Pseudomonas aeruginosa* colonization in cystic fibrosis by early treatment. *Lancet* **338**, 725-726

van Belkum, A., Sluijter, M., de Groot, R., Verbrugh, H., Hermans, P.W. 1996. Novel BOX repeat PCR assay for high-resolution typing of *Streptococcus pneumoniae* strains. *Journal of Clinical Microbiology* **34**, 1176-1179

van Laer, F., Raes, D., Vandamme, P., Lammens, C., Sion, J.P., Vrints, C., Snoek, J., Goossens, H. 1998. An outbreak of *Burkholderia cepacia* with septicaemia on a cardiology ward. *Infection Control and Hospital Epidemiology* **19**, 112-113

van Netton, P., Perales, I., van de Moosdijk, A., Curtis, G.D., Mossel, D.A. 1989. Liquid and solid selective differential media for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp. *International Journal of Food Microbiology* **8**, 299-316

Van Pelt, C., Verduin, C.M., Goessens, W.H.F., Vos, M.C., Tummler, B., Segonds, C., Reubsaet, F., Verbrugh, H., van Belkum, A. 1999. Identification of *Burkholderia spp.* in the clinical microbiology laboratory: comparison of conventional and molecular methods. *Journal of Clinical Microbiology* **37**, 2158-2164

Vandamme, P., Pot, B., Gillis, M., de Vos, P., Kersters, K., Swings, J. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiology Reviews* **60**, 407-438

Vandamme, P., Holmes, B., Vancanneyt, M., Coenye, T., Hoste, B., Coopman, R., Revets, H., Lauwers, S., Gillis, M, Kersters, K., Govan, J.R.W. 1997. Occurance of multiple genomovars of *Burkholderia cepacia* in Cystic Fibrosis patients and proposal *of Burkholderia multivorans* sp. nov. *International Journal of Systematic Bacteriology* **47**, 1188-2000

Vandamme, P., Mahenthiralingam, E., Holmes, B., Coeyne, T., Hoste, B., De Vos, P. 2000. Identification and population structure of *Burkholderia stabilis* sp. Nov. (formerly *Burkholderia cepacia* Genomovar IV). *Journal of Clinical Microbiology* **38**, 1042-1047

Vandamme, P., Henry, D., Coeyne T, Nzula, S., Vancanneyt, M., LiPuma, J.J., Speert, D.P., Govan, J.R.W., Mahenthiralingam E. 2002. *Burkholderia anthina* sp. nov. and *Burkholderia pyrrocinia*, two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular diagnostic tools. *FEMS Immunology and Medical Microbiology* **33**, 143-149

Vandamme, P., Holmes, B., Coenye, T., Goris, J., Mahenthiralingam, E., LiPuma, J.J., Govan, J.R. 2003. *Burkholderia cenocepacia* sp. Nov. – a new twist to an old story. *Research in Microbiology* **154**, 91-96

Vermis, K., Coenye, T., Mahenthiralingam, E., Nelis, H.J., Vandamme, P. 2002. Evaluation of species-specific *recA*-based PCR tests for genomovar level identification within the *Burkholderia cepacia* complex. *Journal of Medical Microbiology* **51**, 937-940

Vermis, K., Vandamme, P.A., Nelis, H.J. 2003. *Burkholderia cepacia* complex genomovars: utilization of carbon sources, susceptibility to antimicrobial agents and growth on selective media. *Journal of Applied Microbiology* **95,** 1191-1199

Versalovic, J., Koeuth, T., Lupski, J.R. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research* **25**, 6823-6831

Viallard, V., Poirier, I., Cournoyer, B., Haurat, J., Wiebkin, S., Ophel-Keller, K., Balandreau, J. 1998. *Burkholderia graminis* sp. nov. a *rhizospheric Burkholderia* species, and reassessment of *(Pseudomonas) phenazinium, (Pseudomonas) pyrrocinia*, and *(Pseudomonas) glathei* as *Burkholderia. International Journal of Systematic Bacteriology* **48**, 549-563

Vincent, M., Figueredo, H., Neu, C. 1988. Synergy of ciprofloxacin with fosfomycin *in vitro* against *Pseudomonas* isolates from patients with cystic fibrosis. *Journal of Antimicrobial Chemotherapy* **22**, 41-50

Warren, L.S., Benoit, R.E., Jessee, J.A. 1978. Rapid enumeration of fecal coliforms in water by a colorimetric ß-galactosidase assay. *Applied and Environmental Microbiology* **35**, 136-141

Wasilauskas, B.L. 1971. Preliminary observations on the rapid differentiation of the *Klebsiella-Enterobacter-Serratia* group on bile-esculin-agar. *Applied Microbiology* **21**, 162-163

Watt, V.M., Yip, C.C. 1989. Amino acid sequence deduced from a rat kidney cDNA suggests it encodes the Zn-peptidase aminopeptidase *The Journal of Biological Chemistry* **264**, 5480-5487

Wauters, G., Boel, A., Voorn, G.P., Verhaegen, J., Meunier, F., Janssens, M., Verbist, L. 1995. Evaluation of a New Identification System, Crystal Enteric/Non-Fermenter, for Gram –Negative Bacilli. *Journal of Clinical Microbiology* **33**, 845-849

Webb, A.K., Dodd, M.E. 1997. Nebulised antibiotics for adults with cystic fibrosis. *Thorax* **52**, 69-71

Weiss, K. and Lapointe, J.R. 1995. Routine susceptibility testing of four antibiotic combinations for improvement of laboratory guide to therapy of cystic fibrosis infections caused by *Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy* **39**, 2411-2414

Welch, D.F., Muszynski, M.J., Pai, C.H., Marcon, M.J., Hribar, M.M., Gilligan, P.H., Matsen, J.M., Ahlin, P.A., Hilman, B.C., Chartrand, S.A. 1987. Selctive and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. *Journal of Clinical Microbiology* **25**, 1730-1734

Werneburg, B., Monteil, H., 1989. New serotypes of *Pseudomonas cepacia*. *Research in Microbiology* **140**, 17-20.

Westmacott, D., Hall, M., Hassal, C., Lees, L., Rowe, B., Ward, L. 1981. Activity of alafosfalin against bacterial pathogens of the gastrointestinal tract *in vitro* and *in vivo*. *Proceedings of 12th International Congress of Chemotherapy*. Florence, **I**, 340-342.

Westley, J.R., Anderson, P.J., Close, V.A., Halburn, B., Lederberg, E.M. 1967. Aminopeptidase profiles of various bacteria. *Applied microbiology* **15**, 822-825

Whitby, P.W., Carter, K.B., Hatter, K.L., LiPuma, J.J., Stull, T.L 2000. Identification of members of the *Burkholderia cepacia* complex by species-specific PCR. *Journal of Clinical Microbiology* **38**, 2962-2965

White, R.L., Burgess, D.S., Manduru, M., Bosso, J.A. 1996. Comparison of three different *in vitro* methods of detecting synergy: time kill, chequerboard, and E-test. *Journal of Clinical Microbiology* **40**, 1914-1918

Wigfield, S.M., Rigg, G.P., Kavari, M., Webb, A.K., Matthews, R.C., Burnie, J.P. 2002. Identification of an immunodominant drug efflux pump in *Burkholderia cepacia*. *Journal of Antimicrobial Chemotherapy* **49**, 619-624.

Wilkinson, S.G., Pitt, T.L., 1995. *Burkholderia (Pseudomonas) cepacia*: surface chemistry and typing methods. *Reviews in Medical Microbiology* **6**, 1-9.

Witt, I. 1977. New methods for the analysis of coagulation using chromogenic substrates, de Gruyter, Berlin.

Wooton, M., Hedges, A.J., Bowker, K.E., Holt, H.A., Reeves, D.S., MacGowan, A.P. 1995. A critical assessment of the agar dilution chequerboard technique for studying *in vitro* antimicrobial interactions using a representative beta-lactam, aminoglycoside and fluoroquinolone. *Journal of Antimicrobial Chemotherapy* **35**, 569-576

Wright, R.C. 1984. A new selective and differential agar medium for *Escherichia coli* and coliform organisms. *Journal of Applied Bacteriology* **56**, 381-388

Wu, B.J., Thompson, S.T. 1984. Selective medium for *Pseudomonas cepacia* containing 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan and polymyxin B sulphate. *Applied and Environmental Microbiology* **48**, 743-746

Yabuuchi, E., Kosako, Y. 1992. Proposal of *Burkholderia* gen nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus with the type species *Burkholderia cepacia* comb. Nov., *Microbiology and Immunology* **36**, 1251-1257

Yabuuchi, E., Kawamura, Y., Ezaki, T., Ikedo, M., Dejsirilert, M., Fujiwara, N., Naka, T., Kobayashi, K. 2000. *Burkholderia ubonensis* sp. nov., L-arabinose-assimilating but different from *Burkholderia thailandensis* and *Burkholderia vietnamiensis*. *Microbiology Immunology* **44**, 307-317

You, M.S., Facklam, R.R. 1986. New test for identification of *Aerococcus*, *Enterococcus* and *Streptococcus*. *Journal of Clinical Microbiology* **24**, 607-611

Yu, V.L., Zuravleff, J.J., Peacock, J.E., DeHertogh, D., Tashjian, L. 1984. Addition of rifampin to carboxypenicillin-aminoglycoside combination for the treatment of *Pseudomonas aeruginosa* infection: clinical experience with four patients. *Antimicrobial Agents and Chemotherapy* **26**, 575-577

Yu, W.L., Wang, D.Y., Lin, C.W., Tsou, M.F. 1999. Endemic *Burkholderia cepacia* bacteraemia: clinical features and antimicrobial susceptibilities of isolates. *Scandinavian Journal of Infectious Diseases* **31**, 293-298

Zimmerman, M., Yurewicz, E., Patel, G. 1976. A new fluorogenic substrate for chymotrpysin. *Analytical Biochemistry* **70**, 258-262

Zuravleff, J.J., Chervenick, P., Yu, V.L., Muder, R.R., Diven, W.F. 1984. Addition of rifampin to ticarcillin-tobramycin combination for the treatment of *Pseudomonas aeruginosa* infections: assessment in a neutropenic mouse model. *Journal of Laboratory and Clinical Medicine* **103**, 878-885

Appendices

Appendix 2.1: Microbanked Bcc strains isolated from patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit upon which *recA* PCR-RFLP analysis was performed

Strain no.	Reference	Patient	Specimen type
W1	2451	A	Sputum
W2	917	B	Sputum
W3	918	B	Sputum
W4	1335	B	Sputum
W5	1598	B	BAL
W6	1599	B	BAL
W7	1604	B	BAL
W8	1637	B	BAL
W9	1968	B	BAL
W10	2060	B	Sputum
W11	2390	B	Sputum
W12	2729	B	Sputum
W13	864	C	Sputum
W14	865	C	BAL
W15	869	C	Sputum
W16	881	C	Sputum
W17	887	C	Sputum
W18	891	C	Sputum
W19	1987	D	Sputum
W20	1944	E	Sputum
W21	2324	E	Sputum
W22	2529	E	BAL
W23	2565	E	Sputum
W24	2567	E	BAL
W25	2587	E	Sputum
W26	2595	E	BAL
W27	2784	E	BAL
W28	2384	F	Sputum
W29	2795	G	Sputum
W30	1331	H	Sputum
W31	2330	I	Sputum
W32	1488	J	Sputum
W33	703	K	Sputum
W34	775	K	Sputum
W35	2455	K	Sputum
W36	1148	L	Sputum
W37	1150	L	BAL
W38	1160	L	Sputum

Appendix 2.1 (cont'd..): Microbanked Bcc strains isolated from patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit upon which *recA* PCR-RFLP analysis was performed

Strain no.	Reference	Patient	Specimen type
W39	1166	L	Sputum
W40	1168	L	BAL
W41	1169	L	Pleural aspirate
W42	1170	L	Pleural aspirate
W43	1171	L	Pleural aspirate
W44	1172	L	Pleural aspirate
W45	1176	L	Sputum
W46	1177	L	Sputum
W47	1178	L	BAL
W48	1179	L	BAL
W49	1181	L	Sputum
W50	1201	L	Sputum
W51	1202	L	Sputum
W52	1203	L	Sputum
W53	1204	L	Sputum
W54	1205	L	Sputum
W55	2295	M	Sputum
W56	2396	M	Sputum
W57	2916	N	Sputum
W58	1791	O	Sputum
W59	2604	P	Sputum
W60	2430	Q	Sputum
W61	2432	Q	Sputum
W62	2433	Q	Sputum
W63	2449	Q	Sputum
W64	2450	Q	Sputum
W65	2454	Q	BAL
W66	2574	Q	Sputum
W67	2577	Q	Sputum
W68	2830	Q	BAL
W69	2141	Q	Sputum
W70	411	R	Sputum
W71	413	R	Sputum
W72	2674	S	Sputum
W73	2686	T	Sputum
W74	1112	U	Sputum
W75	1206	U	Pleural aspirate
W76	1210	U	Sputum
W77	1211	U	BAL

Appendix 2.1 (cont`d..): Microbanked Bcc strains isolated from patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit upon which *recA* PCR-RFLP analysis was performed

Strain no.	Reference	Patient	Specimen type
W78	1217	U	BAL
W79	2232	V	Sputum
W80	2683	W	Sputum
W81	136	X	Sputum
W82	2642	Y	Sputum
W83	2643	Y	Sputum
W84	2679	Y	BAL
W85	2680	Y	BAL
W86	2738	Y	BAL
W87	374	Z	Sputum
W88	650	Z	Sputum
W89	651	Z	Sputum
W90	659	Z	Sputum
W91	660	Z	Sputum
W92	661	Z	BAL
W93	2458	Z	Sputum
W94	2528	Z	Sputum
W95	1063	AA	Sputum
W96	2991	AB	Sputum
W97	294294	AC	Sputum
W98	291171	AD	Sputum
W99	294253	AE	Sputum
W100	2952795	AF	Sputum
W101	320628	AG	Sputum
W102	314270	AH	Sputum
W103	313814	AI	Sputum
W104	37463	AJ	Sputum
W105	333874	AK	Sputum
W106	344958	AL	Sputum
W107	341566	AM	Sputum
W108	322107	AN	Sputum
W109	S48	AO	Sputum
W110	S51	AP	Sputum
W111	356892	AQ	Sputum
W112	367323	AR	Sputum

(BAL: bronchoalveolar lavage)

Appendix 2.2: Genotypes of wild Bcc isolates upon which *recA* PCR-RFLP analysis was performed

Strain no.	Patient	Genotype	Strain no. (cont`d..)	Patient	Genotype
W1	A	III-A	W37	L	III-A
W2	B	V	W38	L	III-A
W3	B	V	W39	L	III-A
W4	B	V	W40	L	III-A
W5	B	V	W41	L	III-A
W6	B	V	W42	L	III-A
W7	B	V	W43	L	III-A
W8	B	V	W44	L	III-A
W9	B	V	W45	L	III-A
W10	B	V	W46	L	III-A
W11	B	V	W47	L	III-A
W12	B	V	W48	L	III-A
W13	C	III-A	W49	L	III-A
W14	C	III-A	W50	L	III-A
W15	C	III-A	W51	L	III-A
W16	C	III-A	W52	L	III-A
W17	C	III-A	W53	L	III-A
W18	C	III-A	W54	L	III-A
W19	D	II	W55	M	III-A
W20	E	V	W56	M	III-A
W21	E	V	W57	N	II
W22	E	V	W58	O	II
W23	E	V	W59	P	III-A
W24	E	V	W60	Q	II
W25	E	V	W61	Q	II
W26	E	V	W62	Q	II
W27	E	V	W63	Q	II
W28	F	II	W64	Q	II
W29	G	III-B	W65	Q	II
W30	H	*A. xylosoxidans*	W66	Q	II
W31	I	*A. xylosoxidans*	W67	Q	II
W32	J	II	W68	Q	II
W33	K	II	W69	Q	II
W34	K	II	W70	R	II
W35	K	II	W71	R	II
W36	L	III-A	W72	S	III-A

Appendix 2.2 (cont`d..): Genotypes of wild Bcc isolates upon which *recA* PCR-RFLP analysis was performed

Strain no.	Patient	Genotype
W79	V	III-A
W80	W	III-A
W81	X	*B. vesicularis*
W82	Y	II
W83	Y	II
W84	Y	II
W85	Y	II
W86	Y	II
W87	Z	II
W88	Z	II
W89	Z	II
W90	Z	II
W91	Z	II
W92	Z	II
W93	Z	II
W94	Z	II
W95	AA	III-A
W96	AB	III-A
W97	AC	III-A
W98	AD	III-A
W99	AE	III-A
W100	AF	III-B
W101	AG	III-B
W102	AH	III-B
W103	AI	II
W104	AJ	II
W105	AK	III-A
W106	AL	II
W107	AM	III-B
W108	AN	II
W109	AO	III-B
W110	AP	III-A
W111	AQ	III-A
W112	AR	III-A

Appendix 2.3: PFGE analysis results on wild Bcc strains isolated from patients referred to the Freeman Hospital Cardiopulmonary Transplant Unit

Strain no.	Patient	Genotype	Pre/post transplant	PFGE type
W1	A	III-A	unknown	unique
W2	B	V	pre	cluster 1
W6	B	V	post	cluster 1
W13	C	III-A	pre	ET-12
W16	C	III-A	post	ET-12
W19	D	II	pre	unique
W20	E	V	pre	Cluster 2
W23	E	V	post	Cluster 2
W28	F	II	pre	unique
W29	G	III-B	unknown	unique
W30	H	non-Bcc	pre	unique
W31	I	non-Bcc	pre	unique
W32	J	II	pre	unique
W33	K	II	unknown POST	cluster 3
W35	K	II	pre	cluster 3
W37	L	III-A	post	ET-12
W55	M	III-A	pre	ET-12
W56	M	III-A	post	ET-12
W57	N	II	pre	unique
W58	O	II	pre	unique
W59	P	III-A	unknown	ET-12
W60	Q	II	unknown	cluster 4
W65	Q	II	unknown	cluster 4
W70	R	II	unknown	unique
W72	S	III-A	pre	ET-12
W73	T	III-A	pre	ET-12
W74	U	III-A	unknown	ET-12
W75	U	III-A	unknown PRE	ET-12
W76	U	III-A	post	ET-12
W79	V	III-A	unknown	ET-12
W80	W	III-A	post	ET-12
W81	X	non-Bcc	pre	unique
W82	Y	II	post	unique
W91	Z	II	post	unique
W95	AA	III-A	unknown	ET-12
W96	AB	III-A	unknown	ET-12
W97	AC	III-A	unknown	ET-12
W98	AD	III-A	unknown	ET-12
W99	AE	II	post	unique
W100	AF	III-B	unknown	unique

Appendix 3.1: References for *P. aeruginosa* strains isolated from pre-operative broncho alveolar lavages (BAL) of CF lung transplant patients attending the Freeman Hospital Cardiopulmonary Transplantation Unit

Strain no.	Reference
1	2688
2	2702
3	2704
4	2706
5	2715
6	2720
7	2737
8	2739
9	2741
10	2742
11	2749
12	2772
13	2775
14	2776
15	2778
16	2779
17	2780
18	2781
19	2782
20	2783

Appendix 3.2a: Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	1	2	3	4	5	6	7	8	9	10
P. aeruginosa	2688	24	-19	89	-49	15	-70	-40	55	25	265
P. aeruginosa	2702	3	-9	65	-48	-3	-61	-40	64	241	89
P. aeruginosa	2704	3	12	98	-31	0	-58	-40	61	83	86
P. aeruginosa	2706	27	-15	98	-43	-15	-62	-46	58	13	67
P. aeruginosa	2715	3	-6	86	-22	-27	-33	-40	52	28	147
P. aeruginosa	2720	61	-18	22	-46	18	-58	-49	67	24	159
P. aeruginosa	2737	70	131	101	-49	0	-37	-58	116	46	1221
P. aeruginosa	2739	49	-27	55	-58	-12	-64	-61	58	16	193
P. aeruginosa	2741	12	-9	55	-36	-22	-73	-34	79	98	61
P. aeruginosa	2742	55	-18	76	-36	0	-94	-37	55	64	162
P. aeruginosa	2749	12	-52	39	-52	0	-73	-31	64	333	131
P. aeruginosa	2772	27	-22	165	-34	-9	-70	-24	79	67	153
P. aeruginosa	2775	3	-27	46	-43	3	-62	-21	24	3	534
P. aeruginosa	2776	45	1736	385	-34	113	-64	-19	86	9	254
P. aeruginosa	2778	164	39053	54194	-33	4678	9	-52	824	92	11174
P. aeruginosa	2779	-9	-9	125	-37	9	-68	-31	64	6	1303
P. aeruginosa	2780	232	14292	12886	-9	0	3827	-34	70	-3	302
P. aeruginosa	2781	9	-31	122	-31	-19	-34	-58	55	15	144
P. aeruginosa	2782	15	-19	76	-61	6	-58	-46	51	12	131
P. aeruginosa	2783	18	-40	98	-46	-34	-30	-24	64	22	82
P. aeruginosa	PS1	-12	4	104	-15	0	-58	-24	61	9	689
P. aeruginosa	PS2	12	6	83	-49	13	-27	-12	91	58	192
P. aeruginosa	PS3	-3	-25	67	-49	-33	-58	-37	43	52	58
P. aeruginosa	PS4	34	-30	83	-25	12	-46	-28	58	73	180
P. aeruginosa	PS5	34	-25	134	-40	18	-19	-15	67	10	501
P. aeruginosa	PS6	46	-43	52	-43	-12	-12	-43	58	10	82
P. aeruginosa	PS7	22	58	85	-43	6	-73	-30	122	19	110
P. aeruginosa	PS8	27	-22	95	-34	-9	-55	-55	82	25	265
P. aeruginosa	PS9	3	-12	104	9	-6	-37	-27	91	101	424
P. aeruginosa	PS10	34	-31	77	-58	-7	-74	-34	61	49	242
P. aeruginosa	PS11	9	-9	91	-49	-25	-89	-52	61	12	321
P. aeruginosa	PS12	3	-22	83	-7	-7	-86	-46	88	100	137
P. aeruginosa	PS13	122	412	565	-33	15	-82	-46	205	80	119
P. aeruginosa	PS14	6	-31	74	-34	-22	-71	-55	55	16	418
P. aeruginosa	PS15	113	281	92	-37	-16	-74	-67	140	116	165
P. aeruginosa	PS16	9	-22	76	-58	0	-40	-52	52	33	247
P. aeruginosa	PS17	9	-40	98	-37	3	-76	-43	55	10	183
P. aeruginosa	PS18	18	-21	70	-37	-18	-85	0	73	110	140
P. aeruginosa	PS19	-3	-28	24	-24	-3	-74	-34	70	7	196
P. aeruginosa	PS20	168	598	110	-31	-22	-48	-46	293	-6	146
P. aeruginosa	PS21	-16	-25	70	-21	-31	-70	-25	64	7	320
P. aeruginosa	PS22	15	-19	76	-64	-19	-73	-34	76	3	155
P. aeruginosa	PS23	192	574	83	-52	-15	-86	-34	275	28	103
P. aeruginosa	PS24	9	-16	162	-43	13	-64	-22	89	-13	193
P. aeruginosa	PS25	18	7	95	-40	22	-77	-40	67	0	135
P. aeruginosa	PS26	52	-22	107	-19	-24	-86	-34	73	22	943
P. aeruginosa	PS27	16	-34	70	-28	-16	-73	-25	58	-13	158
P. aeruginosa	PS28	18	-55	104	-24	3	-61	-28	88	153	302

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	1	2	3	4	5	6	7	8	9	10
P. aeruginosa	PS29	55	287	77	-61	-6	-80	-70	140	147	31
P. aeruginosa	PS30	18	-49	58	-39	0	-65	-49	58	40	79
P. aeruginosa	PS31	34	131	113	-49	-9	-70	-52	110	49	18
P. aeruginosa	PS32	30	-31	91	-55	-6	-94	-28	55	46	183
P. aeruginosa	PS33	9	-7	180	-24	-16	-67	-40	58	15	1554
P. aeruginosa	PS34	-6	-37	64	-30	30	-61	-49	18	3	205
P. aeruginosa	PS35	6	-49	76	-67	-25	-89	-40	64	15	34
P. aeruginosa	PS36	42	-40	70	-37	12	-67	-19	70	-9	119
P. aeruginosa	PS37	27	-25	83	-49	-13	-49	-40	30	3	122
P. aeruginosa	PS38	88	226	64	-43	-15	-58	-46	156	19	122
P. aeruginosa	PS39	116	232	98	-55	-18	-61	-28	113	42	109
P. aeruginosa	PS40	3	-19	67	-61	-18	-58	-58	55	58	70
P. aeruginosa	PS41	-3	6	189	-34	31	-34	3	86	37	485
P. aeruginosa	PS42	18	190	125	-46	13	-82	-31	88	25	64
P. aeruginosa	PS43	-24	15	174	-21	-9	-70	-28	76	34	250
P. aeruginosa	PS44	34	-9	143	-30	-9	-82	-22	94	46	140
P. aeruginosa	PS45	-3	16	168	-55	0	-77	-6	52	18	192
P. aeruginosa	PS46	31	6	137	-33	-19	-80	-61	52	6	400
P. aeruginosa	PS47	107	302	144	-39	-18	-64	-22	168	18	281
P. aeruginosa	PS48	21	-7	134	-30	-16	-31	-3	68	-6	263
P. aeruginosa	PS49	18	-25	113	-55	-12	-70	-21	88	49	122
P. aeruginosa	PS50	15	28	149	-37	-3	-58	-21	85	24	125
P. aeruginosa	PS51	18	46	204	6	33	-43	-24	100	58	650
P. aeruginosa	PS52	27	-12	150	-52	15	-73	-27	64	55	824
B. cepacia	LMG 1222	193	41934	52583	-15	17359	-37	0	2112	125	15434
B. cepacia	LMG 2161	226	33423	49485	-24	2786	-64	39666	1049	86	23695
B. cenocepacia	LMG 16654	141	39233	53294	-22	12800	-55	-21	1221	73	9198
B. cenocepacia	LMG 16656	214	39523	53172	-18	12986	-64	-28	1318	55	10654
B. cenocepacia	LMG 16659	265	31197	41632	-46	2511	-55	41009	1004	67	27880
B. cepacia	LMG 17997	9119	36038	53287	3	5658	6256	18932	921	110	34516
B. cepacia	LMG 18821	208	2023	53303	-21	2087	-31	23848	680	37	19259
B. cenocepacia	LMG 18826	106	13459	53376	-4	1251	-61	20311	463	34	24791
B. cenocepacia	LMG 18827	135	982	177	848	3467	48	82	1105	49	13197
B. cenocepacia	LMG 18828	137	33099	42768	-22	2542	-65	-55	427	238	30562
B. cenocepacia	LMG 18829	305	40118	53016	-27	14262	-37	-12	1519	95	25014
B. cenocepacia	LMG 18830	128	17695	37406	-16	2411	-46	-43	351	28	27639
B. cenocepacia	LMG 18832	391	9110	53459	-58	2747	-22	49	1078	67	22282
B. cenocepacia	LMG 18863	446	12040	54753	-9	3009	-25	45	1184	83	22722
B. multivorans	LMG 13010	177	2176	38639	-25	3641	-12	-9	824	34	23110
B. multivorans	LMG 16660	314	39286	54164	6	4199	85	85	937	43	21703
B. multivorans	LMG 16665	262	674	12373	-28	3125	186	274	915	31	16038
B. multivorans	LMG 17588	128	668	116	-37	3645	-52	-31	964	18	18874
B. multivorans	LMG 18822	58	143	110	-22	1736	-55	-43	372	9	22484
B. multivorans	LMG 18823	110	787	287	-31	2872	-46	-12	918	131	17323
B. multivorans	LMG 18824	146	226	196	-37	1600	55	159	485	25	11699
B. multivorans	LMG 18825	107	88	171	-55	839	-86	-28	265	46	8231
B. stabilis	LMG 14086	156	623	54121	-37	1358	155	40811	217	86	13001
B. stabilis	LMG 14294	195	601	235	-28	4327	49	141	1306	116	15391

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	1	2	3	4	5	6	7	8	9	10
B. stabilis	LMG 18870	289	38919	40512	-28	16384	-3	-58	-55	52	21342
B. stabilis	LMG 18888	-94	1023	41413	-3	1278	-9	40537	28	31	14179
B. vietnamiensis	LMG 10929	220	58	241	-21	793	-15	-61	125	-46	21953
B. vietnamiensis	LMG 16232	-67	70	28	-12	695	-18	-101	6	-39	10051
B. vietnamiensis	LMG 18835	-61	64	43	-24	790	-3	-79	52	-55	10029
B. vietnamiensis	LMG 18836	778	43	41242	18	882	6	-76	85	-33	13130
A. baumannii	ATCC 19606	-129	-61	55	-16	-19	0	-43	-95	-33	247
A. calcoaceticus	7844	-128	-55	46	-13	-31	-21	-67	-95	-24	373
A. haemolyticus	12155	-122	-49	58	-15	-31	-18	-64	-131	-37	287
A. johnsonii	10308	-134	-55	45	-12	-79	-18	-67	-110	-30	379
A. lwoffi	5866	-113	473	5491	-31	296	-15	296	-70	10	293
A. lwoffi	5867	-141	-49	107	-52	-52	-21	-113	-101	-21	247
A. lwoffi	NCIMB 12456	67	794	858	22	159	808	803	-113	-28	174
B diminuta	ATCC 11568	-128	-34	1630	-24	-58	-24	-64	-98	-55	15254
B. vesicularis	ATCC 11426	15	18398	7	-28	36	3477	-67	-58	0	13358
R. pickettii	11149	-138	-36	95	-27	-61	-21	-58	-95	80	906
C. meningosepticum	ATCC 13253	1645	18007	18431	110	165	31179	4819	-98	387	16387
M. nonliquefaciens	10464	174	1917	1010	-12	-34	3394	824	-83	-33	247
M. osloensis	10465	116	1691	5634	0	70	6885	1138	-70	22	814
M. urethralis	11010	-122	-49	43	-22	-67	15	-110	-112	-6	73
O. urethralis	11999	43	1779	1426	-9	-37	6571	748	-113	-10	382
P. acidovorans	10683	-122	-37	15	-34	-30	-9	-104	-104	16	1001
P. aeruginosa	6749	-116	33	40	-31	-52	-9	-73	-122	-24	2857
P. aeruginosa	10332	-91	3	43	15	-25	31	-58	-64	137	437
P. alcaligenes	10367	363	3	28	-43	-67	3061	-70	-85	22	3968
P. pseudoalcaligenes	10860	-125	58	177	-6	-67	24	-58	-67	357	2735
P. diminuta	8545	-104	-55	3269	-18	-42	0	-73	-76	-30	28946
P. fluorescens	10754	-131	-27	67	-21	-39	-15	-52	-61	-36	312
P. fluorescens	10392	360	4111	1913	-15	-43	4929	1383	-79	-21	610
P. fluorescens	3756	-104	-27	64	-25	-64	-9	-40	-101	-46	137
P. fluorescens	10038	-119	-55	76	-10	-55	-30	-49	-95	-40	3971
P. fluorescens	10688	-104	-37	79	-34	-79	21	-86	-107	-40	659
P. fluorescens	9428	-70	-70	58	-31	-73	-21	-46	-76	-18	1010
P. fragi	NCIMB 8987	35486	846	895	-3	424	1038	21197	1379	-21	12297
P. maltophilia	10257	-116	19029	5185	-6	-7	2838	43	-113	3690	33944
P. paucimobilis	11030	8973	21465	13740	31	5558	15791	4236	15739	516	18284
R. pickettii	11149	-58	-12	91	-25	-58	-3	-46	-74	-9	260
P. putida	10936	-140	-21	101	-22	-40	-27	-58	-95	-55	497
P. stutzeri	12262	-131	2023	3980	-25	-55	4499	-6	-101	-42	790
P. stutzeri	10475	-143	-34	141	-28	-65	919	-70	-104	-43	107
P. vesiculare	10900	12	27685	-22	-3	214	3336	-73	-73	37	12904
S. spiritivorum	ATCC 33861	7437	20381	13267	91	4581	25014	19503	507	11156	18843
B. ambifaria	11351	125	2191	41699	3	2380	15	-49	592	-40	3311
B. andropogonis	1279	1159	116	143	-49	-21	229	46	3418	-18	1126
B. andropogonis	2126	20555	382	626	-28	183	479	11179	711	-21	415
B. caryphylli	2155	12	-58	16	-6	589	0	-40	-70	479	4135
B. caryphylli	2156	-76	-30	64	-15	314	3	-46	-98	-40	2942
B. dolosa	18941	-52	3	42744	15	687	-9	-61	94	-48	7511

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	1	2	3	4	5	6	7	8	9	10
B. dolosa	18942	-55	95	43052	6	2378	-4	-52	358	-36	12748
B. gladioli	11626	-119	-18	33957	10	52	-34	-43	-79	-64	1652
B. gladioli	18113	-110	76	38861	13	391	-12	-46	-67	-52	3761
B.gladioli pv. alliicola	2121	-76	293	43274	28	967	-6	-6	-18	-39	1682
B. gladioli pv. alliicola	6877	-42	629	38400	6	164	1890	183	-55	24	5332
B.gladioli pv. gladioli	2216	-110	104	31872	24	351	-33	-52	-52	-15	2796
B.gladioli pv. gladioli	6880	-79	284	42124	9	1291	-37	-6	-24	6	7877
B. glumae	1277	16	903	28	-3	7776	-25	30276	1139	-45	3806
B. glumae	2196	-21	-21	27	0	76	-18	27733	-64	-42	3152
B. phenazinium	2247	-110	4053	3550	-37	-37	52	-45	-110	262	24309
B. phenazinium	6868	131	1441	2414	15	-12	3922	888	-55	-43	19084
P. apista	16408	-128	-21	192	-6	-51	-18	-82	-113	16	1739
P. norimberensis	13019	-122	-31	49	-34	-43	-42	-73	-85	-45	498
P. norimberensis	16603	-98	-9	34	-19	-51	-21	-67	-101	-52	342
P. pnomenusa	18087	-107	-40	73	-31	-43	-12	-82	-86	-61	205
P. pnomenusa	18817	-107	-31	27	-3	-55	-21	-70	-95	-64	98
P. pulmonicola	18107	-116	-70	43	-25	-45	-24	-82	-101	-42	1752
P. sputorum	18100	-113	-49	21	-12	-58	-27	-88	-79	-39	55
P. sputorum	18819	-128	-28	9	-15	-45	-12	-88	-95	-73	101
R. basilensis	18990	-110	-58	18	-31	-37	-12	-24	-89	-39	2515
R. basilensis	19286	-107	-39	12	-3	-21	-34	-70	-95	-40	3223
R. campinensis	19282	-134	-52	27	45	-55	-12	-46	-98	-61	580
R. campinensis	19283	-113	-40	31	-9	-70	-9	-67	-95	-33	876
R. eutropha	1190	-113	-34	95	3	-67	3	-46	-110	-33	12404
R. eutropha	1194	-110	-40	91	-34	-55	0	-82	-101	-46	11143
R. gilardii	3399	-104	216	31	-19	-9	98	-76	-125	-30	5829
R. gilardii	3400	-107	226	24	-25	-22	85	-76	-79	-36	3776
R. mannitolilytica	19090	-119	385	27	-9	0	64	-49	-74	-48	299
R. metallidurans	1195	-125	-34	31	2078	-45	235	-76	-107	-43	766
R. metallidurans	19290	-131	-40	58	-28	-58	-3	-40	-198	9	684
R. paucula	3244	-131	-55	119	-6	-61	9	-28	-101	-45	110
R. paucula	3245	-132	-46	40	6	-61	-24	-64	-104	-27	106
R. pickettii	5942	-85	-43	61	-3	-21	-9	-76	-122	-39	171
R. pickettii	6871	-109	-58	40	-6	-52	-12	-85	-113	-34	811
R. solanacearum	2291	162	-43	321	-6	-67	201	-27	-116	-39	348
R. solanacearum	2293	-73	-49	34	-18	-64	-36	-18	-92	-34	167
R. taiwanensis	19425	-88	1187	73	-61	-30	2017	375	-110	-27	4392
S. maltophilia	957	-94	15883	8183	-21	119	20849	1825	-128	9492	40887
S. maltophilia	958	-49	15019	9550	-21	-7	739	49	-122	9202	37527
H_2O		-95	-34	-22	-27	-40	-9	-92	-110	-30	49
H_2O		-85	-61	12	-22	-46	-18	-98	-107	-52	21
H_2O		3	-13	-15	10	-46	-3	-79	-101	-48	55
H_2O		-73	-51	6	27	-36	-15	-103	-116	-45	36
H_2O		-76	-58	-6	3	-45	-27	-79	-112	-43	36
E. coli	10418	29281	110	58	31686	-52	266	2414	116	-27	5668
Enterobacter cloacae	11936	26473	580	1655	27	38617	85	1590	287	43	1593

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	11	12	13	14	15	16	17	18	19	20
P. aeruginosa	2688	952	25130	11482	5087	18089	2499	8335	6512	27	20427
P. aeruginosa	2702	535	20164	19835	5152	5067	6073	16280	6946	79	19555
P. aeruginosa	2704	738	22600	10621	6074	5527	6351	10828	6971	58	19801
P. aeruginosa	2706	873	21355	16799	5619	6107	5082	16343	6958	64	20491
P. aeruginosa	2715	565	22105	11424	6129	5622	2637	19365	6684	76	20027
P. aeruginosa	2720	507	24220	33920	4264	10246	-491	7920	6443	24	19862
P. aeruginosa	2737	1026	23397	22490	9107	8387	-250	19442	6821	74	19035
P. aeruginosa	2739	852	20231	14442	7936	6507	-73	18950	6260	18	20562
P. aeruginosa	2741	446	22924	25844	7212	2857	2091	13743	6565	12	19854
P. aeruginosa	2742	650	23091	22121	7736	4257	3644	33575	6598	45	20525
P. aeruginosa	2749	781	20485	19469	6132	6388	2643	16478	6620	24	20186
P. aeruginosa	2772	1786	21520	5146	7984	4032	4499	17006	6782	61	19954
P. aeruginosa	2775	1627	19851	5427	6584	24	3241	14586	6934	67	20201
P. aeruginosa	2776	721	23229	7465	11345	5902	2939	19753	6922	95	20635
P. aeruginosa	2778	626	21514	6150	5066	19905	3613	13951	6626	171	33032
P. aeruginosa	2779	504	21254	29159	4111	7206	2005	29366	6662	98	21095
P. aeruginosa	2780	372	17991	15068	5442	6181	2320	15562	7136	3601	20650
P. aeruginosa	2781	278	22100	6836	7566	3601	2634	9047	6324	-6	20094
P. aeruginosa	2782	476	22902	30770	8549	5344	2286	27785	6327	61	21190
P. aeruginosa	2783	891	19572	34780	7175	2906	3077	33612	6513	58	21532
P. aeruginosa	PS1	348	22301	20144	6961	3672	2188	15098	6522	0	21599
P. aeruginosa	PS2	479	24722	9822	9638	4633	4208	12076	6400	58	21300
P. aeruginosa	PS3	391	22655	23913	5756	5154	5140	22154	7795	31	21504
P. aeruginosa	PS4	458	22344	15968	7175	5994	6049	13829	7163	3	20128
P. aeruginosa	PS5	3312	21727	14955	7206	6168	1444	12293	6305	177	20607
P. aeruginosa	PS6	513	21380	16688	6080	4154	6931	8869	6479	18	19878
P. aeruginosa	PS7	415	24315	18532	7801	7701	5255	8473	6744	61	20641
P. aeruginosa	PS8	693	22783	28832	6809	5402	10338	24709	7173	22	20928
P. aeruginosa	PS9	565	26998	22554	13520	16112	8387	12449	6654	25	20031
P. aeruginosa	PS10	831	23180	18096	6828	4023	6364	14579	6986	37	20656
P. aeruginosa	PS11	913	25203	18513	7212	10569	2744	17970	6593	76	20903
P. aeruginosa	PS12	336	22613	13856	7596	5060	10145	10874	6635	15	20842
P. aeruginosa	PS13	727	22939	22319	8942	7514	6275	20125	7505	46	20076
P. aeruginosa	PS14	690	24352	12934	7858	15300	4883	10511	6592	30	20159
P. aeruginosa	PS15	1230	24055	20131	9906	9553	7368	17223	7468	12	21129
P. aeruginosa	PS16	507	22969	17580	7453	3876	4068	19030	6968	12	20296
P. aeruginosa	PS17	1071	22230	10005	7422	7868	3421	15617	6825	16	19948
P. aeruginosa	PS18	302	22420	17497	7148	6436	6974	11818	7688	-9	20091
P. aeruginosa	PS19	375	25579	20391	11329	4258	4670	21660	7402	-15	19869
P. aeruginosa	PS20	846	23775	13728	9864	10157	2982	18001	7167	98	20723
P. aeruginosa	PS21	797	23455	5988	7597	2644	3763	9705	6922	27	20710
P. aeruginosa	PS22	309	26580	20052	7343	8698	7300	15608	7508	-3	21001
P. aeruginosa	PS23	666	26080	13966	8949	7618	6010	15504	7704	24	20412
P. aeruginosa	PS24	833	23839	34893	8051	7489	1755	23037	6687	49	19651
P. aeruginosa	PS25	437	22866	9559	8982	6544	3452	13933	6489	10	20446
P. aeruginosa	PS26	754	24810	17644	8142	7816	9065	9815	6846	89	20336
P. aeruginosa	PS27	620	24285	7237	9510	9922	3513	10667	6660	6	19609
P. aeruginosa	PS28	406	22990	11421	8408	4037	6400	14967	7771	25	20689

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	11	12	13	14	15	16	17	18	19	20
P. aeruginosa	PS29	1428	20210	9709	5374	4743	4633	10450	7575	46	20140
P. aeruginosa	PS30	956	19817	17515	6220	3827	2210	14616	6934	71	20265
P. aeruginosa	PS31	329	23684	14656	6205	4996	6052	8027	7758	-9	20430
P. aeruginosa	PS32	955	23638	13233	7139	8558	5600	13264	7031	-6	19942
P. aeruginosa	PS33	552	22661	7954	8662	11610	1889	5747	6828	27	20479
P. aeruginosa	PS34	1102	22786	13011	8270	7248	6565	21236	7649	34	20739
P. aeruginosa	PS35	516	19743	-979	4371	1623	4105	274	6632	40	19380
P. aeruginosa	PS36	278	22740	27465	5866	4077	7453	10634	7472	-6	20235
P. aeruginosa	PS37	1206	20342	11912	6846	2569	857	23101	6842	49	20500
P. aeruginosa	PS38	232	21855	25680	6733	4752	6113	16859	7624	-24	20427
P. aeruginosa	PS39	580	24209	21648	7706	4877	5106	17796	7280	9	20577
P. aeruginosa	PS40	125	15156	25634	3748	784	2005	25460	6595	-12	19997
P. aeruginosa	PS41	1954	24916	19368	9562	2890	4252	16273	6949	79	20931
P. aeruginosa	PS42	678	427	19023	6223	4404	6373	12614	7935	-6	20702
P. aeruginosa	PS43	1456	24468	11051	8717	9095	3952	10066	6699	76	20741
P. aeruginosa	PS44	861	24462	16990	8921	3999	4639	17223	7157	76	20504
P. aeruginosa	PS45	897	23537	15098	7551	5463	3769	16108	7053	110	20518
P. aeruginosa	PS46	275	20546	16469	3619	6324	3396	7947	6577	27	20995
P. aeruginosa	PS47	659	24803	26864	7712	8991	3107	16651	7236	6	20448
P. aeruginosa	PS48	558	23705	39222	7562	11832	3903	31683	6809	27	20183
P. aeruginosa	PS49	455	24892	21507	7096	7746	9619	17744	7438	36	19960
P. aeruginosa	PS50	400	23299	20388	7816	4474	3833	18269	6867	94	20842
P. aeruginosa	PS51	357	24114	24938	8818	5811	4236	15718	6885	82	20918
P. aeruginosa	PS52	391	22640	18944	7654	6891	2551	17305	6806	-37	21001
B. cepacia	LMG 1222	296	23375	2390	5130	21660	13087	32663	6803	1095	37188
B. cepacia	LMG 2161	653	28814	5396	13593	11259	31030	20180	7133	143	24285
B. cenocepacia	LMG 16654	427	23635	2923	3891	16298	2975	36270	6870	757	36080
B. cenocepacia	LMG 16656	339	23351	2662	3934	15831	3290	36700	7025	391	35821
B. cenocepacia	LMG 16659	489	23610	6192	11362	7463	22036	21052	8564	183	26564
B. cepacia	LMG 17997	684	24880	6714	5350	16301	32776	31604	8650	415	34060
B. cepacia	LMG 18821	449	20341	2112	4947	2090	17545	3135	7340	107	20863
B. cenocepacia	LMG 18826	510	16346	16813	4975	772	24126	4761	7676	147	20930
B. cenocepacia	LMG 18827	537	25784	16453	9382	34897	21114	14320	8939	61	20799
B. cenocepacia	LMG 18828	177	24535	4816	10007	9449	28295	15574	7502	229	34546
B. cenocepacia	LMG 18829	525	22710	7337	5787	17759	19057	40308	8069	260	23912
B. cenocepacia	LMG 18830	251	20992	3922	7895	7706	29366	7608	8522	434	21678
B. cenocepacia	LMG 18832	2652	23292	5225	12693	4346	29132	12834	8249	339	22860
B. cenocepacia	LMG 18863	599	25207	5948	15236	5933	32519	20363	8298	113	22136
B. multivorans	LMG 13010	543	23412	3967	10749	7111	34812	12708	8058	91	20330
B. multivorans	LMG 16660	556	21511	3400	8485	4748	24206	21440	9510	253	20583
B. multivorans	LMG 16665	827	23747	5066	11216	4013	36429	7954	8826	82	20393
B. multivorans	LMG 17588	610	24974	22045	9415	35977	19521	48621	8851	107	20793
B. multivorans	LMG 18822	952	24685	16621	9253	33966	20699	48496	7215	107	19960
B. multivorans	LMG 18823	693	24120	18040	10294	35675	23724	47526	8332	125	20250
B. multivorans	LMG 18824	668	23272	18108	7331	5988	33160	26784	8729	89	21355
B. multivorans	LMG 18825	852	25841	46177	13810	5716	32897	26577	8143	67	20281
B. stabilis	LMG 14086	1633	23464	6891	16859	6333	32961	10840	8018	110	20864
B. stabilis	LMG 14294	733	23671	4499	10349	32544	22221	10743	8781	40	20265

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	11	12	13	14	15	16	17	18	19	20
B. stabilis	LMG 18870	781	15071	7587	7078	21257	21385	40231	7533	1542	29607
B. stabilis	LMG 18888	2735	19658	12284	16740	7239	26260	12288	7242	250	21056
B. vietnamiensis	LMG 10929	3745	20589	10862	19912	7337	22429	19920	9989	13	27221
B. vietnamiensis	LMG 16232	2231	17603	11121	7905	5726	23833	17888	7258	0	20308
B. vietnamiensis	LMG 18835	1450	15962	5592	6525	2939	8420	13865	7050	-21	20915
B. vietnamiensis	LMG 18836	2240	19750	9085	22365	2896	5949	11241	7536	0	21392
A. baumannii	ATCC 19606	5994	13490	16767	3474	6504	13288	25155	8835	37	21233
A. calcoaceticus	7844	1819	17690	17741	5076	13380	37118	28653	9709	46	21437
A. haemolyticus	12155	723	12156	27053	3543	5319	20461	39973	9485	27	21129
A. johnsonii	10308	751	12504	14344	3290	5237	12122	7541	7719	18	21483
A. lwoffi	5866	1346	8323	14467	2939	6259	4017	18761	7743	312	21669
A. lwoffi	5867	602	7785	4336	2502	4666	21806	10865	7013	22	21425
A. lwoffi	NCIMB 12456	2130	8659	43707	2490	7398	2533	14887	7593	28	21800
B diminuta	ATCC 11568	775	12989	30266	5973	37104	25985	41547	9199	30	33129
B. vesicularis	ATCC 11426	738	12605	36463	8457	39954	16490	24880	7602	4422	32764
R. pickettii	11149	742	19600	30038	4318	3213	11124	8307	8802	-6	23302
C. meningosepticum	ATCC 13253	473	20525	47971	7267	35732	37634	41410	20391	1782	39249
M. nonliquefaciens	10464	406	17988	18077	8430	7691	4511	14763	6446	70	22087
M. osloensis	10465	138	14992	12284	3437	15748	16344	8500	14039	-33	30477
M. urethralis	11010	418	15611	34246	7252	5042	21981	17375	11030	89	22420
O. urethralis	11999	272	16737	46671	5726	9806	10080	19505	8429	9	27721
P. acidovorans	10683	2668	19069	11812	2811	6498	6858	1343	7474	34	23870
P. aeruginosa	6749	2039	18761	26223	8381	4364	4456	10587	6363	16	21080
P. aeruginosa	10332	1089	19447	18315	10289	11811	4251	12571	6857	89	20937
P. alcaligenes	10367	186	5381	25039	2533	2255	1352	12135	8625	-12	22551
P. pseudoalcaligenes	10860	604	15376	16975	8082	10905	1138	32532	6537	107	25115
P. diminuta	8545	580	9816	7209	8134	37408	21406	40487	8985	-9	34891
P. fluorescens	10754	897	19661	35046	16486	32346	2286	19923	7080	104	21721
P. fluorescens	10392	519	19535	26516	8240	8661	2762	10957	6889	39	21486
P. fluorescens	3756	2063	17658	34267	5921	4493	2390	19374	6506	-15	21605
P. fluorescens	10038	375	19093	23125	6797	6439	2753	6465	6507	3	21284
P. fluorescens	10688	1050	19279	40680	9282	19759	1035	10154	7016	-9	20714
P. fluorescens	9428	852	17335	22511	6162	24605	778	10316	6559	-9	21602
P. fragi	NCIMB 8987	378	22252	18554	4148	2878	1850	11854	6379	-18	21514
P. maltophilia	10257	220	12556	26219	3239	1136	1642	6083	6422	12867	37063
P. paucimobilis	11030	1615	20229	47963	4233	812	4095	41656	11125	15602	37149
R. pickettii	11149	595	18922	30807	4007	4487	8012	2585	8955	52	21876
P. putida	10936	940	17329	40167	8911	37280	580	25298	7233	34	22399
P. stutzeri	12262	2747	20189	20781	16984	14732	1361	40070	7602	46	22090
P. stutzeri	10475	647	19197	12910	10591	8949	681	34070	8106	58	21855
P. vesiculare	10900	528	11988	39807	8109	39108	13499	20393	8079	3235	32168
S. spiritivorum	ATCC 33861	687	19004	40518	2859	19133	23280	28283	13802	13514	31496
B. ambifaria	11351	199	19289	5264	8451	24898	9800	34380	7630	52	26806
B. andropogonis	1279	1004	20414	28927	6366	5597	19762	11348	8799	-24	22276
B. andropogonis	2126	610	18559	8585	4294	4761	11005	10346	8741	89	21834
B. caryphylli	2155	870	21345	39740	14653	23122	39465	26488	10358	-15	23744
B. caryphylli	2156	907	20121	18376	11647	14137	33575	11711	11714	70	21290
B. dolosa	18941	622	18205	10945	9128	5591	27880	13460	8191	-3	22872

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	11	12	13	14	15	16	17	18	19	20
B. dolosa	18942	388	19816	13814	11930	21035	24077	27599	7721	-13	22936
B. gladioli	11626	1215	15553	30752	9449	12984	2939	22319	7538	28	22319
B. gladioli	18113	2994	17638	23668	8439	13087	20873	22564	8231	28	22020
B.gladioli pv. alliicola	2121	3165	17991	21789	8924	13840	5045	17680	6959	363	22466
B. gladioli pv. alliicola	6877	528	20710	13942	15559	20604	18291	20302	7740	9	21700
B.gladioli pv. gladioli	2216	3055	20332	23503	18065	30768	22444	27450	8412	21	22524
B.gladioli pv. gladioli	6880	2549	20513	20269	24565	32010	20369	19340	7246	-7	22350
B. glumae	1277	677	18559	27980	9614	29394	8830	44281	7072	58	20925
B. glumae	2196	781	20720	19668	12379	25329	19307	28332	7486	19	21413
B. phenazinium	2247	858	21327	26906	14759	38016	42444	13621	8815	-6	35891
B. phenazinium	6868	964	19954	19017	18538	27489	37097	7676	8835	80	22130
P. apista	16408	375	19447	10182	3244	-1285	10615	922	7551	3	17949
P. norimberensis	13019	522	19487	23760	4385	500	8155	1047	7517	6	21685
P. norimberensis	16603	522	17384	16847	4114	1121	6217	1123	6702	15	20461
P. pnomenusa	18087	549	20287	22649	5970	4474	26979	1053	8570	-3	20940
P. pnomenusa	18817	390	17369	18410	4483	2079	9083	1297	7761	12	21666
P. pulmonicola	18107	482	11295	14262	3861	-97	9565	2008	7309	-3	19985
P. sputorum	18100	2081	14808	19777	7071	940	12324	1184	6980	-27	21297
P. sputorum	18819	778	19970	21086	9052	2909	16111	15437	8387	3	20570
R. basilensis	18990	1493	18193	17201	5967	2027	8881	965	7588	-37	22036
R. basilensis	19286	473	20619	13313	13588	3690	12388	1481	7379	-9	22237
R. campinensis	19282	610	18669	37610	6278	4423	23405	5000	8268	-12	22148
R. campinensis	19283	455	19310	41113	7880	7801	29732	7289	8377	24	21367
R. eutropha	1190	1315	19216	29477	11967	7679	21120	9165	9318	10	21490
R. eutropha	1194	323	18929	31005	10709	6565	17101	8720	7972	-18	21575
R. gilardii	3399	656	19225	34464	8142	10584	8256	3351	7435	13	21468
R. gilardii	3400	607	19404	11552	6888	7593	10737	1410	7242	28	21810
R. mannitolilytica	19090	2945	19667	9028	16908	4413	24877	4034	7752	16	21804
R. metallidurans	1195	366	18919	15980	9086	3022	12352	2353	7197	-24	21800
R. metallidurans	19290	968	20314	27541	13371	5292	12062	2234	7474	-6	22084
R. paucula	3244	492	18690	22621	6763	4269	20861	2472	9028	-12	21449
R. paucula	3245	626	20647	33898	10350	7346	21385	1095	8402	-12	21666
R. pickettii	5942	1047	20369	40366	5777	6086	16041	6418	7899	9	21981
R. pickettii	6871	1542	21059	23998	19993	3894	23073	9669	7639	-15	21691
R. solanacearum	2291	220	17830	41376	5176	2072	3333	18681	7420	18605	21007
R. solanacearum	2293	2524	19097	40967	12459	2860	1227	6797	7499	39	21987
R. taiwanensis	19425	1743	18206	24382	6818	4303	3955	1761	7099	49	21742
S. maltophilia	957	705	17598	36023	3775	4771	1773	4556	7633	3519	38724
S. maltophilia	958	382	10923	9876	3290	1597	1679	2561	6855	12874	36700
H_2O		49	2301	2097	2466	647	375	174	6720	-31	21367
H_2O		18	2612	2115	2375	669	464	42	6666	-51	21087
H_2O		18	2408	2008	2396	641	464	168	6675	-24	21242
H_2O		30	2441	2237	2381	718	351	98	6440	-48	21218
H_2O		24	2475	2161	2292	604	360	198	6724	-36	21288
E. coli	10418	507	20308	8689	10611	4639	2713	31844	6898	0	20146
Enterobacter cloacae	11936	3321	19784	15428	5137	5182	2851	30117	6675	281	20116

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	21	22	23	24	25	26	27	28	29
P. aeruginosa	2688	-589	41392	53505	-64	876	-1520	41742	2136	-400
P. aeruginosa	2702	-629	47895	55012	-49	620	-1642	65966	2182	-52
P. aeruginosa	2704	-657	46418	56038	-40	949	-1404	3757	1856	37
P. aeruginosa	2706	-568	50599	56056	-85	1782	-1160	67736	2484	183
P. aeruginosa	2715	-608	51539	55836	-58	678	-1312	35095	2371	-100
P. aeruginosa	2720	-619	45572	39105	-65	308	-1374	36344	2756	-110
P. aeruginosa	2737	-638	51042	53468	-76	1480	-1203	36234	3708	196
P. aeruginosa	2739	-608	54231	53642	-52	1431	-1194	54466	2784	-272
P. aeruginosa	2741	-641	49748	55839	-51	595	-1285	52623	3028	446
P. aeruginosa	2742	-879	52986	55580	-82	403	-1340	67870	2698	82
P. aeruginosa	2749	-596	56105	54579	-40	857	-1303	36191	3080	-189
P. aeruginosa	2772	-586	56639	53093	-68	391	-1459	65123	2173	-150
P. aeruginosa	2775	-568	37597	51878	-70	149	-1157	36026	2069	-296
P. aeruginosa	2776	-561	49336	55052	-46	284	-1398	23360	2289	-286
P. aeruginosa	2778	-540	53941	52595	-24	89	-1688	1434	2439	-378
P. aeruginosa	2779	-552	49592	55168	-86	104	-1798	34417	5506	-287
P. aeruginosa	2780	-553	49052	55977	-55	247	-1471	42593	2570	-131
P. aeruginosa	2781	-565	50661	56642	-74	159	-1627	54200	2063	-196
P. aeruginosa	2782	-550	57372	56484	-80	201	-1706	46909	4078	95
P. aeruginosa	2783	-582	53932	55281	-51	103	-1575	56645	4331	104
P. aeruginosa	PS1	-580	48875	54679	-58	40	-1719	39206	2783	-345
P. aeruginosa	PS2	-613	49848	56764	-21	86	-1770	38135	2366	134
P. aeruginosa	PS3	-494	52525	54921	-58	77	-1718	68087	2613	818
P. aeruginosa	PS4	-611	53004	54295	-64	12	-1737	68185	2414	293
P. aeruginosa	PS5	-785	44782	52345	-82	125	-1276	34011	2182	-119
P. aeruginosa	PS6	-552	40970	54017	-73	46	-1507	66045	2637	204
P. aeruginosa	PS7	-595	46589	54142	-46	85	-1675	68313	2612	634
P. aeruginosa	PS8	-607	49671	56828	-67	147	-1600	67953	3119	19
P. aeruginosa	PS9	-666	44437	56804	-27	92	-1517	68471	2747	-186
P. aeruginosa	PS10	-580	50132	56966	-80	64	-1373	66481	2707	-113
P. aeruginosa	PS11	-619	56254	56987	-64	55	-1544	48237	3052	-189
P. aeruginosa	PS12	-586	48328	55269	-61	89	-1657	68160	2340	546
P. aeruginosa	PS13	-631	50941	56062	-61	49	-1709	68703	2790	354
P. aeruginosa	PS14	-620	49668	53925	-65	64	-1550	36749	2243	-299
P. aeruginosa	PS15	-586	50254	55827	-61	113	-1728	66494	2213	43
P. aeruginosa	PS16	-583	53394	54546	-43	140	-1584	60588	2408	-141
P. aeruginosa	PS17	-565	42658	50279	-22	92	-1251	62640	3308	-18
P. aeruginosa	PS18	-534	46693	55532	-61	92	-1688	68094	2780	321
P. aeruginosa	PS19	-516	47935	55754	-62	241	-1383	43378	3132	-250
P. aeruginosa	PS20	-626	49742	57441	-68	42	-1462	55894	2796	263
P. aeruginosa	PS21	-562	49637	56630	-46	92	-1620	64181	2164	-143
P. aeruginosa	PS22	-599	45924	57090	-61	98	-1681	67315	2716	-101
P. aeruginosa	PS23	-601	51323	55265	-61	180	-1382	69714	2301	772
P. aeruginosa	PS24	-683	45255	42499	-45	82	-1184	51642	5619	100
P. aeruginosa	PS25	-629	50113	57411	-36	52	-1144	33160	2591	-357
P. aeruginosa	PS26	-741	55540	57807	-45	131	-1272	69299	2826	-137
P. aeruginosa	PS27	-586	50792	54270	-61	27	-1221	60046	2439	-33
P. aeruginosa	PS28	-579	51304	56291	-55	51	-1523	67938	2490	98

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	21	22	23	24	25	26	27	28	29
P. aeruginosa	PS29	-549	43253	50571	-40	204	-1258	56078	2148	211
P. aeruginosa	PS30	-583	40567	51282	-39	177	-1258	36386	2695	-216
P. aeruginosa	PS31	-476	43677	53666	-55	119	-1437	58439	2009	25
P. aeruginosa	PS32	-516	43210	55178	-61	168	-1282	62026	2091	-46
P. aeruginosa	PS33	-528	48442	52626	-49	156	-1355	39279	1986	-290
P. aeruginosa	PS34	-543	48506	55000	-74	256	-1477	65478	2982	202
P. aeruginosa	PS35	-604	49894	52705	-58	238	-1377	47703	1999	-390
P. aeruginosa	PS36	-546	54090	55080	-70	135	-1465	66851	3119	128
P. aeruginosa	PS37	-586	49788	55659	-68	122	-1206	23906	3019	-168
P. aeruginosa	PS38	-543	50422	56517	-74	150	-1474	66964	2905	202
P. aeruginosa	PS39	-568	53025	56132	-43	125	-1362	58314	2673	92
P. aeruginosa	PS40	-690	43629	51130	-79	52	-1361	61012	2027	-259
P. aeruginosa	PS41	-540	49317	53038	-33	3	-1431	21928	2636	-269
P. aeruginosa	PS42	-470	44220	53828	-31	-12	-1606	65240	2020	2017
P. aeruginosa	PS43	-583	49430	56920	-30	85	-1682	52950	2274	156
P. aeruginosa	PS44	-540	52803	55711	-39	58	-1813	68432	2615	428
P. aeruginosa	PS45	-629	52852	41052	-37	122	-1551	22686	3027	-165
P. aeruginosa	PS46	-583	50728	58147	-65	37	-1642	25869	2469	-351
P. aeruginosa	PS47	-528	48265	55440	-58	40	-1559	48087	2854	315
P. aeruginosa	PS48	-626	58256	55205	-113	202	-1410	33111	8149	-137
P. aeruginosa	PS49	-647	50114	56541	-55	132	-1602	65932	2844	626
P. aeruginosa	PS50	-662	49229	55626	-36	146	-1297	21697	2502	-9
P. aeruginosa	PS51	-541	49345	58065	-19	73	-1367	46684	2280	-150
P. aeruginosa	PS52	-537	50978	54951	-18	12	-1426	50196	2851	-232
B. cepacia	LMG 1222	-445	53258	54057	183	153	-1301	3132	2332	-351
B. cepacia	LMG 2161	-513	51408	54341	-18	-6	-1434	12156	2741	-275
B. cenocepacia	LMG 16654	-537	52949	57905	77	79	-1523	5390	2356	-146
B. cenocepacia	LMG 16656	-553	55379	57988	91	46	-1477	5408	2350	-168
B. cenocepacia	LMG 16659	-531	55095	57203	16	107	-1301	4618	2829	-195
B. cepacia	LMG 17997	-540	56865	58068	98	528	-1480	6354	4343	470
B. cepacia	LMG 18821	-553	63457	56288	-21	49	-1557	5237	1987	-52
B. cenocepacia	LMG 18826	-586	55626	56676	22	134	-1413	3616	2286	-186
B. cenocepacia	LMG 18827	-622	50636	57591	37	-7	-1214	1575	2649	-89
B. cenocepacia	LMG 18828	-534	60732	56658	-33	67	-1447	4156	2033	-64
B. cenocepacia	LMG 18829	-495	57069	58791	18	403	-1206	5909	2744	-332
B. cenocepacia	LMG 18830	-519	61156	57496	-36	86	-1407	2047	2140	-388
B. cenocepacia	LMG 18832	-494	56795	55269	55	385	-1502	5475	2322	-79
B. cenocepacia	LMG 18863	-504	56062	54899	58	89	-1199	4849	3120	-128
B. multivorans	LMG 13010	-506	51634	55442	-15	2200	-1123	1889	2204	-302
B. multivorans	LMG 16660	-513	54405	56007	46	296	-1181	3876	2115	-131
B. multivorans	LMG 16665	-562	55870	56132	-3	849	-1138	2743	2173	-152
B. multivorans	LMG 17588	-552	57988	55968	-28	388	-1431	1407	4624	-25
B. multivorans	LMG 18822	-525	56532	53917	-58	363	-1370	1160	3519	4846
B. multivorans	LMG 18823	-546	58333	54612	-15	1422	-1520	2145	4367	-217
B. multivorans	LMG 18824	-547	55692	55891	-9	55	-1132	2368	2673	67
B. multivorans	LMG 18825	-567	55104	55751	-55	363	-1248	2292	2911	27
B. stabilis	LMG 14086	-577	51591	53157	-6	162	-1398	11311	2368	-112
B. stabilis	LMG 14294	-488	51243	55482	22	58	-1260	1062	1782	-192

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	21	22	23	24	25	26	27	28	29
B. stabilis	LMG 18870	-132	46369	58134	174	980	-213	1956	6543	326
B. stabilis	LMG 18888	-220	51246	57787	19	2643	-131	4102	14433	552
B. vietnamiensis	LMG 10929	-241	50264	57789	-19	2179	46	61	14182	1162
B. vietnamiensis	LMG 16232	-204	49848	57725	-22	1038	-162	9	5543	455
B. vietnamiensis	LMG 18835	-201	48417	58913	-4	846	-61	364	3742	431
B. vietnamiensis	LMG 18836	-229	50034	58329	16	1556	-58	-4	11262	495
A. baumannii	ATCC 19606	-122	47300	52211	-28	2183	58	55	8683	1083
A. calcoaceticus	7844	-149	44812	54035	-9	721	82	650	9263	2579
A. haemolyticus	12155	-147	45497	55855	-25	641	184	28	39322	1941
A. johnsonii	10308	-190	45059	54814	-13	631	-24	25	14012	901
A. lwoffi	5866	-131	48868	51277	0	2801	-55	378	4145	1395
A. lwoffi	5867	-91	47593	54320	-46	4508	-137	10	2192	1260
A. lwoffi	NCIMB 12456	-223	40506	49162	-22	195	-149	260	18339	1578
B diminuta	ATCC 11568	-226	48823	56383	-16	272	238	2139	43729	909
B. vesicularis	ATCC 11426	-269	50388	50083	-6	415	-80	8832	40497	30089
R. pickettii	11149	-164	51393	59904	-6	238	156	949	10230	875
C. meningosepticum	ATCC 13253	793	44452	49549	-27	1034	1691	33	46207	52479
M. nonliquefaciens	10464	-314	50306	59462	9	287	134	27001	35938	393
M. osloensis	10465	-183	49607	54228	-13	266	632	5692	13426	452
M. urethralis	11010	-238	51430	35772	-22	373	269	815	26036	629
O. urethralis	11999	-232	48038	39697	-16	290	332	756	28805	632
P. acidovorans	10683	-208	48597	58809	15	393	-77	250	1627	183
P. aeruginosa	6749	-226	49845	58245	-3	283	48	33202	40369	360
P. aeruginosa	10332	-140	47987	59135	52	345	-101	32111	37304	394
P. alcaligenes	10367	-226	48521	52845	-10	299	-3	3229	38174	152
P. pseudoalcaligenes	10860	-226	49457	40274	-6	293	-229	2487	41535	210
P. diminuta	8545	-229	49808	42603	-22	378	122	2139	49198	204
P. fluorescens	10754	21	45124	24263	-6	354	-141	39777	32613	1422
P. fluorescens	10392	-277	49705	36044	-16	390	-152	43134	40641	863
P. fluorescens	3756	-171	43457	28475	-6	280	-86	55021	45139	946
P. fluorescens	10038	-311	50725	50257	-12	329	-89	57216	20351	2191
P. fluorescens	10688	-256	50086	57839	-10	290	-134	53126	30611	657
P. fluorescens	9428	-329	48652	51988	-28	271	-28	54044	15672	485
P. fragi	NCIMB 8987	-213	48872	23873	7502	232	170	7185	13084	522
P. maltophilia	10257	43	50395	56340	-7	870	378	56679	7710	839
P. paucimobilis	11030	-253	49040	55684	3815	940	3153	146	45325	28371
R. pickettii	11149	-226	49387	58891	25	241	107	253	2939	757
P. putida	10936	-256	44981	23390	-34	305	-248	20668	38211	317
P. stutzeri	12262	-250	46326	32117	-3	256	-16	1251	47122	2240
P. stutzeri	10475	-262	52692	56661	-10	302	-229	37	45419	550
P. vesiculare	10900	-207	53266	37613	-16	366	-116	7715	39167	27075
S. spiritivorum	ATCC 33861	-27	45771	59041	4532	1620	1761	543	33365	25652
B. ambifaria	11351	-223	50196	55733	36	309	-141	4584	6568	385
B. andropogonis	1279	-207	52904	49879	1600	364	-46	781	18859	296
B. andropogonis	2126	-229	48450	53670	629	327	-116	201	11200	296
B. caryphylli	2155	-216	49269	56615	24	412	2930	1221	35666	4385
B. caryphylli	2156	-244	50019	58547	30	598	-135	1270	32928	1450
B. dolosa	18941	-217	50563	60542	-13	260	-165	7236	7639	351

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	21	22	23	24	25	26	27	28	29
B. dolosa	18942	-229	52491	59139	-9	296	77	10188	9440	692
B. gladioli	11626	-211	49736	58507	-10	320	-144	2359	42704	1157
B. gladioli	18113	-186	51115	57872	-6	241	33	10017	16017	909
B.gladioli pv. alliicola	2121	-235	56001	58989	-13	275	-22	1583	10142	598
B. gladioli pv. alliicola	6877	-205	49830	59770	-6	277	-21	3027	11045	803
B.gladioli pv. gladioli	2216	-140	55741	59572	-28	202	-22	4929	25213	1681
B.gladioli pv. gladioli	6880	-113	51182	57930	24	210	-158	5335	15190	1788
B. glumae	1277	-9	51426	57301	222	204	-146	44852	10617	8607
B. glumae	2196	-162	49863	55348	15	232	-12	17650	8561	3277
B. phenazinium	2247	-204	51820	49723	-9	232	275	730	15706	425
B. phenazinium	6868	-170	44712	56615	-25	250	-165	18089	17280	1334
P. apista	16408	-311	50682	54036	15	348	-351	540	2072	351
P. norimberensis	13019	-272	49259	44779	3	324	-431	1294	2090	693
P. norimberensis	16603	-251	54106	46754	-46	348	-464	1328	1850	409
P. pnomenusa	18087	-202	49973	54655	-19	299	-201	772	1584	5380
P. pnomenusa	18817	-144	53645	43522	-13	274	-180	6	1981	473
P. pulmonicola	18107	-274	51774	34515	-16	326	-326	589	4755	486
P. sputorum	18100	-189	51316	58663	-16	287	-262	0	2567	443
P. sputorum	18819	-167	45346	34540	-7	360	-262	0	1804	442
R. basilensis	18990	-159	51014	59301	28	241	-150	28	2067	274
R. basilensis	19286	-205	51494	55973	-25	262	-134	31	1981	754
R. campinensis	19282	-186	52137	56178	-28	272	-76	406	3836	445
R. campinensis	19283	-168	44660	57289	-7	309	-91	34	7245	1038
R. eutropha	1190	-187	48256	56523	-15	369	-49	2792	6229	244
R. eutropha	1194	-88	51084	55799	3	378	49	342	7615	281
R. gilardii	3399	-125	48469	56895	-43	360	-162	210	4148	296
R. gilardii	3400	-147	50373	61593	-9	351	-89	977	2536	916
R. mannitolilytica	19090	-196	54893	60381	-49	326	-165	982	4242	663
R. metallidurans	1195	-149	51002	60353	-13	403	-45	-9	4014	332
R. metallidurans	19290	-180	50825	59379	-34	335	-210	-3	2671	293
R. paucula	3244	-174	50736	60368	-16	308	-79	219	3946	430
R. paucula	3245	-180	54350	59951	-19	391	-211	21	1620	693
R. pickettii	5942	-150	51289	58998	6	369	-79	64	6223	650
R. pickettii	6871	-140	52272	58794	-10	451	-128	3	6406	381
R. solanacearum	2291	-225	45765	40222	0	317	-134	2667	33453	635
R. solanacearum	2293	-158	46634	43421	-31	437	-30	2087	5308	555
R. taiwanensis	19425	-205	46451	58730	-9	461	33	4120	2163	183
S. maltophilia	957	165	47200	57524	-22	1019	534	54134	8970	1797
S. maltophilia	958	128	48607	58873	-12	983	220	59175	9260	549
H_2O		-144	37180	9214	22	534	-92	88	909	91
H_2O		-201	32900	10065	-15	751	70	55	949	31
H_2O		-162	36294	10008	-7	623	109	132	922	52
H_2O		-159	34707	9684	0	415	0	36	980	88
H_2O		-184	35372	9833	-43	427	-180	113	1040	61
E. coli	10418	-168	48433	25140	4721	900	64	41755	40738	302
Enterobacter cloacae	11936	-201	49922	24947	3293	717	58	26666	16200	461

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	30	31	32	33	34	35	36	37	38	39
P. aeruginosa	2688	6	10505	272	42875	67266	53776	40555	27892	17921	1996
P. aeruginosa	2702	-28	11671	528	51274	70089	53377	39832	28054	26284	4004
P. aeruginosa	2704	-24	10557	220	40579	57399	34143	40725	30337	22576	2539
P. aeruginosa	2706	-52	12794	897	50743	68926	54268	41565	16637	27477	3079
P. aeruginosa	2715	0	14763	433	50212	69311	54018	40604	9458	32867	2741
P. aeruginosa	2720	-92	11360	867	43278	70574	52232	29571	797	21410	3061
P. aeruginosa	2737	0	17573	2194	59764	69113	53450	36005	6690	28457	5982
P. aeruginosa	2739	-21	12943	693	48670	67559	54692	38781	21392	12654	2902
P. aeruginosa	2741	-33	15736	882	48066	68749	53596	41226	17153	32855	4459
P. aeruginosa	2742	-31	17350	747	50962	66097	52470	41803	9452	29418	3306
P. aeruginosa	2749	-24	13029	886	52674	69443	55107	42862	3687	32921	3718
P. aeruginosa	2772	-12	15877	256	50016	67852	53462	43085	7156	33441	2502
P. aeruginosa	2775	-18	16978	180	44245	69552	51017	20406	1456	27779	2307
P. aeruginosa	2776	-12	16506	406	45050	69500	52980	33920	6208	32802	2640
P. aeruginosa	2778	-42	11689	330	59902	70965	53477	41217	8066	13316	2536
P. aeruginosa	2779	-3	13701	3421	44766	70233	53849	40952	6193	24532	5934
P. aeruginosa	2780	1194	16264	650	54946	69591	54219	37409	6827	31305	2448
P. aeruginosa	2781	-49	11436	-18	47200	71060	53892	35361	1560	28518	1838
P. aeruginosa	2782	3	15788	2103	52519	72149	55184	34243	7499	32742	5622
P. aeruginosa	2783	-24	15895	2500	51198	69546	56099	39392	12748	30788	6507
P. aeruginosa	PS1	22	11491	726	57225	70871	54628	42856	25923	21996	2606
P. aeruginosa	PS2	3	14235	363	51566	71322	54475	37152	17353	29080	2545
P. aeruginosa	PS3	-37	12889	1008	49882	69143	54579	36056	18672	23708	3784
P. aeruginosa	PS4	-31	12610	244	50187	68924	53383	43387	21120	25884	2945
P. aeruginosa	PS5	-10	15302	671	53608	70879	53208	39817	8945	20864	2606
P. aeruginosa	PS6	-34	10306	870	46164	68039	50819	36608	23421	22862	2933
P. aeruginosa	PS7	-28	13242	641	37482	71106	53007	31988	7102	23446	3183
P. aeruginosa	PS8	-34	11680	1458	50184	70464	53733	35721	18004	21758	4550
P. aeruginosa	PS9	-3	12128	1157	51930	69475	51106	35703	16252	21486	4108
P. aeruginosa	PS10	-40	11665	821	46564	69967	55071	40558	20671	25841	3113
P. aeruginosa	PS11	3	14222	1202	56419	69796	52607	39529	8353	24786	2994
P. aeruginosa	PS12	-36	11525	549	46479	71298	52613	34774	14082	23570	2753
P. aeruginosa	PS13	-18	17925	916	45320	70107	54777	39566	21037	36136	5118
P. aeruginosa	PS14	-37	12794	265	45814	69805	54582	30510	11677	18038	2243
P. aeruginosa	PS15	24	14283	821	41303	70214	52070	42420	22856	26665	4260
P. aeruginosa	PS16	-18	11442	299	48179	69662	54094	43400	10154	29406	2783
P. aeruginosa	PS17	-37	14546	1263	51392	69516	52696	40030	9782	23916	2793
P. aeruginosa	PS18	-31	12171	753	46580	71065	54436	39075	27575	21407	3211
P. aeruginosa	PS19	-33	11244	1105	45576	70800	53520	38177	17341	31091	3614
P. aeruginosa	PS20	-6	19023	1038	55036	71448	52452	39590	10801	36572	4475
P. aeruginosa	PS21	-51	14552	339	51475	73569	53294	42294	22033	37225	2216
P. aeruginosa	PS22	-21	10426	610	42265	73450	54170	38614	20500	26430	3384
P. aeruginosa	PS23	-9	16306	528	48136	71283	54396	30218	11457	22426	4340
P. aeruginosa	PS24	-9	14109	3919	41407	70950	53615	17272	1455	11070	4261
P. aeruginosa	PS25	0	15519	876	55922	71133	54246	41608	13654	24810	2292
P. aeruginosa	PS26	-28	12538	809	60622	71084	54606	31882	12590	25002	3171
P. aeruginosa	PS27	-24	15645	733	52036	69524	53435	37478	8839	21386	2276
P. aeruginosa	PS28	-36	13353	860	48225	69940	53251	40772	15532	24724	3138

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	30	31	32	33	34	35	36	37	38	39
P. aeruginosa	PS29	-22	12678	473	36401	70217	53590	40405	30206	25472	3019
P. aeruginosa	PS30	-27	13130	836	31960	70254	54411	27986	6403	20851	3076
P. aeruginosa	PS31	-33	10630	89	35095	70608	54682	36420	23159	27797	3361
P. aeruginosa	PS32	-33	10770	269	42459	71933	55101	34109	14378	17363	2894
P. aeruginosa	PS33	-24	11140	265	58605	71102	55103	38153	14311	14351	1917
P. aeruginosa	PS34	-24	12693	1178	42084	71188	55653	37524	8792	27542	3613
P. aeruginosa	PS35	-36	11015	195	30139	70034	55760	39258	21605	25844	1142
P. aeruginosa	PS36	-30	12641	1413	44367	71279	56150	36606	18083	28664	4165
P. aeruginosa	PS37	-24	17964	1310	59785	72171	55104	30849	11250	22740	2997
P. aeruginosa	PS38	-37	12977	1248	44862	71545	55235	38366	18193	25252	4135
P. aeruginosa	PS39	-13	12294	1056	48930	71222	55281	41019	25045	30081	3714
P. aeruginosa	PS40	-71	10261	373	41943	69985	54759	39041	17790	10920	2191
P. aeruginosa	PS41	3	14292	824	54362	68872	53386	40470	27559	22728	12
P. aeruginosa	PS42	0	12602	455	43384	67226	50333	36453	26366	22591	3934
P. aeruginosa	PS43	-31	18434	516	55687	70464	52623	32061	10410	23696	2420
P. aeruginosa	PS44	13	17256	973	46222	70443	52186	40067	3152	38339	3336
P. aeruginosa	PS45	-21	12849	1428	53236	68243	50746	38666	10715	33508	2888
P. aeruginosa	PS46	-21	12236	451	55204	70938	54851	39765	4756	15782	2848
P. aeruginosa	PS47	-12	19963	1117	54084	67827	54195	32091	11784	18571	5719
P. aeruginosa	PS48	-31	14726	6504	55949	71258	55593	41028	13410	28469	8586
P. aeruginosa	PS49	-40	14994	1047	55302	70043	54511	37817	9831	24486	3455
P. aeruginosa	PS50	-12	16639	637	45499	71906	55137	29156	4871	27288	3714
P. aeruginosa	PS51	-3	12989	980	51320	73218	52253	35562	18870	21474	4221
P. aeruginosa	PS52	-18	13160	1157	60167	70580	52757	42917	25497	12858	2402
B. cepacia	LMG 1222	-18	9388	256	56413	69275	54515	41370	13997	12040	1828
B. cepacia	LMG 2161	-28	10316	501	57145	68917	53294	41687	9202	12119	2170
B. cenocepacia	LMG 16654	-27	9724	247	57878	68984	54601	44917	24679	10594	1712
B. cenocepacia	LMG 16656	-15	10257	431	59468	68679	54844	42793	23803	14186	1740
B. cenocepacia	LMG 16659	-36	10786	617	57377	60247	56404	41846	16793	11902	2564
B. cepacia	LMG 17997	-12	15340	2155	56941	69623	55171	44031	7185	14207	2143
B. cepacia	LMG 18821	-24	9990	-97	55833	67486	55427	42069	5771	18215	1569
B. cenocepacia	LMG 18826	-27	9537	109	58388	68599	56123	43400	14140	8403	2472
B. cenocepacia	LMG 18827	37	11528	918	58349	65843	56019	38610	6210	22326	3445
B. cenocepacia	LMG 18828	-21	10926	186	59276	68474	54951	43604	5772	9712	2197
B. cenocepacia	LMG 18829	-27	10056	580	61363	74212	55534	44688	5780	18330	2505
B. cenocepacia	LMG 18830	-30	8991	40	49940	71814	50916	40106	2268	25002	2038
B. cenocepacia	LMG 18832	-25	12340	168	55007	65282	53141	43872	19908	13951	3006
B. cenocepacia	LMG 18863	-9	12101	1001	56990	66195	53163	44129	20598	14866	3962
B. multivorans	LMG 13010	-9	9730	131	56270	68203	53752	48298	6006	21135	1919
B. multivorans	LMG 16660	6	11487	58	57344	67477	54270	43155	7529	23280	2500
B. multivorans	LMG 16665	34	11872	-19	56471	64703	54628	43137	3345	15504	2454
B. multivorans	LMG 17588	-6	10590	2848	56621	68456	55061	48829	4602	17012	6880
B. multivorans	LMG 18822	0	10572	1574	55006	65008	55537	45594	6565	16423	4660
B. multivorans	LMG 18823	-6	9717	2612	56661	66338	55571	44257	10005	12397	5402
B. multivorans	LMG 18824	-18	12077	714	57786	62486	54418	43906	7752	14576	3061
B. multivorans	LMG 18825	-12	11869	1242	58840	67260	54384	47724	10322	23290	4777
B. stabilis	LMG 14086	58	18614	622	58614	66823	53593	44071	6980	21230	2890
B. stabilis	LMG 14294	-24	10117	57	58745	67083	54082	43488	5937	34875	2173

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	30	31	32	33	34	35	36	37	38	39
B. stabilis	LMG 18870	28	11548	718	55155	70444	55799	44269	6201	25340	1733
B. stabilis	LMG 18888	34	12736	965	55345	62486	52027	43925	17558	16161	2253
B. vietnamiensis	LMG 10929	31	13807	803	56340	64675	56083	42109	11519	25256	2026
B. vietnamiensis	LMG 16232	28	11455	800	58571	67538	54869	46690	2497	23150	1642
B. vietnamiensis	LMG 18835	19	11811	412	56111	65108	56480	46519	2362	19136	1331
B. vietnamiensis	LMG 18836	34	12831	998	58113	70267	54967	41306	15617	34237	2628
A. baumannii	ATCC 19606	30	12522	622	57902	71759	48783	38895	241	24914	1816
A. calcoaceticus	7844	19	13954	477	53120	71682	54286	24095	134	28295	1822
A. haemolyticus	12155	25	14924	1483	55300	69936	52262	41751	2073	46122	3134
A. johnsonii	10308	19	11692	543	56850	71515	47223	40637	5030	15138	2041
A. lwoffi	5866	43	12009	192	55071	65853	52021	42921	6553	12846	3592
A. lwoffi	5867	36	12352	153	54720	65948	51640	43769	3617	40628	1404
A. lwoffi	NCIMB 12456	9	11857	842	55150	70687	52021	42264	12565	9452	2036
B diminuta	ATCC 11568	21	11946	1816	56770	71966	51939	33465	248	17061	5045
B. vesicularis	ATCC 11426	101	15294	1706	56517	69146	54136	36041	391	28543	2539
R. pickettii	11149	18	13044	2008	56574	60188	50309	38568	12373	18370	2524
C. meningosepticum	ATCC 13253	4257	45838	41651	53706	48097	39322	27376	165	41709	38650
M. nonliquefaciens	10464	45	13423	5442	50517	72204	57570	39130	15339	34597	4486
M. osloensis	10465	31	11116	7316	52699	67281	54439	32889	3940	16267	5891
M. urethralis	11010	49	14744	12577	46311	50401	29464	5354	259	9489	5860
O. urethralis	11999	22	13022	21422	30318	34402	19609	20336	1319	9873	7904
P. acidovorans	10683	18	11433	442	38015	70721	53966	20262	-6	8439	1328
P. aeruginosa	6749	30	12981	1377	49067	69781	54634	44162	16493	30804	2652
P. aeruginosa	10332	70	13176	7123	47813	71957	55723	42715	23167	35596	4380
P. alcaligenes	10367	22	9671	8713	52601	68358	30315	10569	445	2903	4972
P. pseudoalcaligenes	10860	34	18275	1194	47422	66296	49641	14864	244	12940	3385
P. diminuta	8545	22	10798	760	51552	66973	47089	16878	241	3101	2100
P. fluorescens	10754	40	19423	937	40576	66241	45905	983	92	34536	3955
P. fluorescens	10392	28	15806	2307	34290	66772	52562	20335	705	42114	2829
P. fluorescens	3756	48	16228	1923	32431	63808	44843	14527	3	27944	3705
P. fluorescens	10038	39	16685	1398	51212	71847	51521	14857	27	38922	2661
P. fluorescens	10688	34	13050	1041	40466	70681	50831	21037	268	27227	2689
P. fluorescens	9428	12	12400	638	40769	68744	52238	23873	12	24951	2069
P. fragi	NCIMB 8987	238	11466	1688	24279	37345	18904	-128	-46	14768	2740
P. maltophilia	10257	131	11540	31524	55400	72253	54573	30694	1501	4807	18120
P. paucimobilis	11030	1166	30840	45890	56690	71182	53602	31564	1148	50929	45416
R. pickettii	11149	34	13075	2076	57341	62145	52332	35998	9214	22496	2012
P. putida	10936	27	15309	2188	24843	49934	35941	7807	125	29733	4920
P. stutzeri	12262	58	34973	4209	31481	63900	41302	10917	622	51710	9849
P. stutzeri	10475	25	23964	1923	29962	68020	49989	19700	403	53825	4655
P. vesiculare	10900	74	14418	2448	52940	70788	54350	9098	43	4490	2637
S. spiritivorum	ATCC 33861	34643	44224	14806	52857	69507	58189	23299	259	24322	16554
B. ambifaria	11351	77	12315	656	59026	70843	56962	45109	2728	21788	2307
B. andropogonis	1279	16	10945	2185	44141	61040	32464	4120	150	15153	3812
B. andropogonis	2126	16	11521	675	43098	58445	32729	3373	6	19911	1783
B. caryphylli	2155	318	20598	11119	56404	70230	55528	44517	4154	49467	16960
B. caryphylli	2156	70	16148	9574	57829	69818	54744	42664	2841	53254	7477
B. dolosa	18941	-3	12021	705	57078	70403	56123	45169	6845	18907	1779

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	30	31	32	33	34	35	36	37	38	39
B. dolosa	18942	-6	12610	724	57827	70135	56590	46253	10016	19939	2359
B. gladioli	11626	34	12184	848	58976	71923	55846	37271	1768	23125	2280
B. gladioli	18113	61	12746	1041	57844	70037	52851	42762	8921	23791	2323
B.gladioli pv. alliicola	2121	16	11750	897	59279	72137	56336	1837	83	2741	2307
B. gladioli pv. alliicola	6877	3	13460	653	56026	70541	55369	49460	5353	51429	1767
B.gladioli pv. gladioli	2216	37	15577	1492	56838	70342	56016	45713	8673	43430	3159
B.gladioli pv. gladioli	6880	40	13505	980	58336	69124	53935	44928	12550	43271	2793
B. glumae	1277	37	13536	1486	53679	61687	50337	42200	14338	13831	12461
B. glumae	2196	7	11985	629	58052	72064	56642	48643	13173	33096	2536
B. phenazinium	2247	12	12016	5936	46537	70315	52586	27663	3357	11744	11738
B. phenazinium	6868	28	12410	772	56645	72324	55098	40958	3022	30465	1792
P. apista	16408	31	10594	532	42749	48402	22567	626	113	27364	1127
P. norimberensis	13019	19	10545	644	47560	49220	26135	-251	73	30486	1749
P. norimberensis	16603	10	11256	259	41760	44092	22280	-317	67	24791	1328
P. pnomenusa	18087	37	11647	656	54698	48448	28149	910	101	30591	1963
P. pnomenusa	18817	15	11430	589	37958	42054	22753	-406	195	22774	1858
P. pulmonicola	18107	-6	11359	577	42630	33609	18202	-824	-37	34057	1862
P. sputorum	18100	25	11897	391	37598	43375	23030	-186	-52	29968	1638
P. sputorum	18819	31	11210	528	41150	58473	33450	-827	-9	12034	1773
R. basilensis	18990	3	11155	665	55198	58498	36725	1285	-19	5664	1218
R. basilensis	19286	25	10670	439	56035	58980	28808	757	7	2438	1242
R. campinensis	19282	22	11262	1236	54491	64299	36368	6339	55	22759	2243
R. campinensis	19283	46	11985	2002	56633	71566	46357	5878	287	16340	2478
R. eutropha	1190	37	11832	1511	53074	63772	43082	17335	1797	19044	2677
R. eutropha	1194	7	11726	1504	49732	56951	34091	13768	1556	10972	2978
R. gilardii	3399	31	11757	690	33703	64491	30199	11985	222	30556	3723
R. gilardii	3400	24	13017	-159	46268	68923	45798	28051	4562	34515	1951
R. mannitolilytica	19090	25	12956	46	58156	70975	55717	8668	748	11048	1913
R. metallidurans	1195	12	11741	476	55940	61516	34073	8686	433	11677	1380
R. metallidurans	19290	19	12001	1206	55751	62161	35147	18879	888	25793	1410
R. paucula	3244	22	12598	1129	58235	74127	53855	34103	4685	26647	1880
R. paucula	3245	24	12462	1752	58855	74865	50233	33068	751	41181	1614
R. pickettii	5942	28	12431	4011	59392	63726	52125	39618	9809	29934	3125
R. pickettii	6871	31	12366	1767	56673	66860	52915	38665	3525	30947	3184
R. solanacearum	2291	37	12815	922	52363	70284	52375	24404	382	27832	4120
R. solanacearum	2293	24	11845	1694	36688	65649	46348	21205	464	37277	5195
R. taiwanensis	19425	22	11054	516	38703	60097	37320	19762	217	7890	2209
S. maltophilia	957	257	11781	14985	55974	69784	54099	27920	678	6653	38550
S. maltophilia	958	184	11806	3403	54094	71655	55189	32898	1593	4117	8091
H_2O		43	11393	-158	19512	54592	31933	-959	238	2610	1120
H_2O		28	11661	-192	18364	53889	30630	-1059	146	2753	1032
H_2O		22	11796	-192	18141	54582	27486	-919	196	2698	1049
H_2O		28	11485	-247	18800	54341	27416	-931	131	2524	1022
H_2O		37	12629	-125	18706	54426	28814	-601	192	2576	1120
E. coli	10418	37	16997	540	29299	50760	25161	2683	12	20488	2451
Enterobacter cloacae	11936	437	11735	198	26885	40475	24904	4727	54	19426	2636

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference										
Strain	**Reference**	40	41	42	43	44	45	46	47	48	49	50
P. aeruginosa	2688	3149	15547	25469	1053	-201	19918	-42	3501	39243	18886	9
P. aeruginosa	2702	10829	15505	29067	4911	592	31280	6	9614	42014	14210	-3
P. aeruginosa	2704	9782	15983	30245	1056	152	345	-36	1334	41586	19667	46
P. aeruginosa	2706	5528	15514	27020	2838	122	32290	-21	5365	42783	16997	25
P. aeruginosa	2715	3885	13722	21504	3199	312	1672	-27	2082	32153	5417	25
P. aeruginosa	2720	248	7652	18834	3687	195	1162	-18	1288	27974	-1074	25
P. aeruginosa	2737	9696	15849	22118	3684	361	1530	-12	4200	16634	-2414	3
P. aeruginosa	2739	-351	13029	20625	2807	113	1584	-18	2244	39911	-3611	0
P. aeruginosa	2741	5591	13704	21498	4691	390	13056	-9	2551	34060	14168	27
P. aeruginosa	2742	-1352	15748	24556	2433	-19	13038	-18	4004	43189	14198	6
P. aeruginosa	2749	-2548	14973	25854	3165	-28	32485	-9	1780	24337	20570	16
P. aeruginosa	2772	3088	14833	22020	4086	202	32555	-46	2045	40692	18752	37
P. aeruginosa	2775	85	271	2262	11555	275	26006	-43	1544	16448	15599	31
P. aeruginosa	2776	3159	11091	21660	6513	708	32440	-45	3272	19283	21398	0
P. aeruginosa	2778	-3955	17286	28506	1425	30	5131	-36	1029	10474	20381	10
P. aeruginosa	2779	-390	13826	24713	2988	189	32000	-27	1687	33896	21922	155
P. aeruginosa	2780	1966	12162	22194	2900	171	31569	-42	3247	17882	21495	3
P. aeruginosa	2781	436	8738	14674	3648	619	13334	-36	1181	31777	21757	16
P. aeruginosa	2782	4499	9852	21577	6369	1153	24487	-42	3278	30765	20643	7
P. aeruginosa	2783	-241	12135	21107	4881	308	32904	-42	3666	25860	21239	3
P. aeruginosa	PS1	573	14302	25039	4142	183	23009	-30	13393	30758	21184	43
P. aeruginosa	PS2	6671	11830	24725	3628	247	33322	-6	12675	17506	20357	52
P. aeruginosa	PS3	-1038	13850	23769	4712	449	28942	-58	3433	42200	20653	24
P. aeruginosa	PS4	1575	15648	27983	5860	509	32491	-30	4721	42362	18333	31
P. aeruginosa	PS5	2659	15468	28826	2469	-12	23721	-15	3455	23738	20241	21
P. aeruginosa	PS6	824	14054	24786	2820	303	34112	-15	9733	44364	19976	9
P. aeruginosa	PS7	-1410	8088	22127	3098	287	34457	-39	797	45057	20778	31
P. aeruginosa	PS8	198	14940	26904	5271	534	33765	-42	9961	43049	20995	25
P. aeruginosa	PS9	3730	10947	25991	1221	3	33905	-88	5573	44306	20424	10
P. aeruginosa	PS10	-952	13804	21831	2493	320	34418	-30	7563	44325	19750	6
P. aeruginosa	PS11	-330	12531	21809	4078	333	34527	-9	3702	38135	20549	12
P. aeruginosa	PS12	4300	12834	25173	3754	437	33593	-48	6455	43417	19313	22
P. aeruginosa	PS13	2426	12452	24667	3592	369	34082	-18	3095	31448	19497	-12
P. aeruginosa	PS14	4166	13026	25542	3666	67	8936	-24	5866	30520	20082	-6
P. aeruginosa	PS15	3269	13166	25146	1599	132	34051	-58	1111	34280	18193	3
P. aeruginosa	PS16	4523	14491	25563	2990	168	34256	-68	6916	42157	21361	25
P. aeruginosa	PS17	342	14686	25258	2323	-104	9745	-33	1990	29028	17595	37
P. aeruginosa	PS18	961	13652	23992	2151	122	34268	-30	8995	44800	20919	46
P. aeruginosa	PS19	-2524	10700	21325	2331	61	34133	-43	5374	40399	21138	21
P. aeruginosa	PS20	-6812	11613	21449	3259	348	33578	-49	1526	23809	21538	19
P. aeruginosa	PS21	-2838	12525	24926	2826	195	19517	-28	4596	31439	20766	28
P. aeruginosa	PS22	1480	14674	27886	3177	321	33770	-49	7868	43882	19090	-12
P. aeruginosa	PS23	-1529	11436	20616	2972	275	33123	-46	2897	40475	17189	21
P. aeruginosa	PS24	-4673	5774	14088	2057	82	10432	-33	1020	23077	7605	25
P. aeruginosa	PS25	4297	13856	23402	2929	-21	29278	-51	4895	25902	21108	7
P. aeruginosa	PS26	1795	14961	27559	2823	290	33032	-15	8521	44740	19407	12
P. aeruginosa	PS27	1395	10941	20684	3274	58	13542	-27	1560	28155	17152	12
P. aeruginosa	PS28	5628	11323	22282	5167	512	25475	-45	6772	43629	19179	18

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference										
Strain	**Reference**	40	41	42	43	44	45	46	47	48	49	50
P. aeruginosa	PS29	3068	14753	27095	2372	375	32064	-21	3391	33920	20497	46
P. aeruginosa	PS30	5533	8582	21181	2427	122	32577	-37	1130	22597	19911	30
P. aeruginosa	PS31	681	8695	20149	2167	476	30523	-27	2338	36581	20366	31
P. aeruginosa	PS32	-1804	10957	21880	1281	137	30257	-27	843	40341	19865	9
P. aeruginosa	PS33	1809	13350	25341	3415	278	22469	-33	7551	29681	19859	9
P. aeruginosa	PS34	2173	12184	23540	3949	403	7313	-21	3976	44208	6739	6
P. aeruginosa	PS35	-2860	14323	25729	2319	284	29162	12	4981	26217	18919	18
P. aeruginosa	PS36	448	9525	19243	2154	190	32397	-21	2439	43332	18150	7
P. aeruginosa	PS37	7215	11579	22972	3305	150	32730	-15	4057	16316	19823	-9
P. aeruginosa	PS38	1182	9974	20836	5008	330	32339	52	9553	41702	19197	24
P. aeruginosa	PS39	34	10954	22466	2875	64	32306	-45	3360	43335	19069	18
P. aeruginosa	PS40	-406	15074	25158	1630	-67	11213	-45	5341	35229	19206	30
P. aeruginosa	PS41	-449	14900	26021	1807	30	31271	-37	7591	13136	21898	83
P. aeruginosa	PS42	-1575	10749	23104	2936	668	32757	-40	8607	38364	20173	27
P. aeruginosa	PS43	2676	8747	24159	2920	217	3	-49	3391	29626	19557	52
P. aeruginosa	PS44	-2484	8204	15880	2842	201	19078	-46	1993	44309	6613	73
P. aeruginosa	PS45	-385	14216	22191	2219	-43	33053	-12	3809	22734	20479	85
P. aeruginosa	PS46	-2170	11140	23424	1303	-159	30846	-3	4294	10087	17769	21
P. aeruginosa	PS47	-3995	9480	22204	3967	925	32809	-18	2994	16649	19325	12
P. aeruginosa	PS48	6232	10291	26287	4599	171	32007	-37	4038	35644	19673	46
P. aeruginosa	PS49	1267	13325	23980	4932	455	32470	-27	7129	43183	19316	55
P. aeruginosa	PS50	1498	9925	24715	4211	613	33141	-33	1458	9571	16246	6
P. aeruginosa	PS51	2988	10783	22783	2634	19	33176	15	2536	17775	16679	30
P. aeruginosa	PS52	629	15040	27157	1441	-186	27361	-21	13657	26671	14588	33
B. cepacia	LMG 1222	13109	16624	27697	4346	-122	589	-27	17644	2305	-4444	23836
B. cepacia	LMG 2161	21020	14225	24474	1660	-116	2451	-42	2679	1437	-2896	15495
B. cenocepacia	LMG 16654	14235	17488	28308	3434	-48	366	-40	18892	873	-4288	13039
B. cenocepacia	LMG 16656	10941	16704	29006	2332	-88	92	-30	15761	1761	-4310	18852
B. cenocepacia	LMG 16659	20955	16172	26281	3287	-162	2548	-43	4240	2298	-5408	21215
B. cepacia	LMG 17997	12501	18034	27803	4785	1004	2054	-27	15849	702	-4785	11237
B. cepacia	LMG 18821	4574	14103	22487	1612	61	2201	-31	14116	818	-3696	7636
B. cenocepacia	LMG 18826	6864	13777	22875	1560	-162	2430	-30	6326	2841	-5366	15834
B. cenocepacia	LMG 18827	2332	14241	26641	18602	-76	3772	-36	9568	168	-3861	5493
B. cenocepacia	LMG 18828	4596	14018	22722	2405	-128	6425	-39	6500	1435	-2720	14195
B. cenocepacia	LMG 18829	12651	16136	26562	4843	-324	977	-36	9082	1532	-3245	12690
B. cenocepacia	LMG 18830	5006	12251	23305	3140	-86	7344	-64	1352	2286	-3494	20207
B. cenocepacia	LMG 18832	601	18391	19630	5463	381	16279	-24	8100	2308	-3809	15895
B. cenocepacia	LMG 18863	-537	16094	24782	4733	-107	4264	-12	12354	2090	-4468	17244
B. multivorans	LMG 13010	-759	15755	25753	4673	-113	5012	-42	7737	2045	-3632	26140
B. multivorans	LMG 16660	4325	16184	26769	5463	42	6711	-68	8021	1722	-4898	21431
B. multivorans	LMG 16665	-1523	16319	26485	2796	-174	1657	-21	13782	1566	-5970	21175
B. multivorans	LMG 17588	-748	28976	27823	36603	275	7169	-27	10509	598	-4443	5621
B. multivorans	LMG 18822	1672	16139	27651	34033	421	5292	-30	7154	-9	-4825	6430
B. multivorans	LMG 18823	815	15410	26638	13615	83	3897	-15	9528	-12	-3760	8127
B. multivorans	LMG 18824	-2640	14567	26391	4807	-241	4975	-30	5960	-6	-3498	1510
B. multivorans	LMG 18825	-2762	12843	24962	5677	-287	7926	-6	2679	-19	-2655	1832
B. stabilis	LMG 14086	8249	16487	28219	2514	-52	10029	-27	2603	3626	-4712	6668
B. stabilis	LMG 14294	2097	15004	27395	1010	-283	31539	-58	2897	2863	-4862	9318

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference										
Strain	**Reference**	40	41	42	43	44	45	46	47	48	49	50
B. stabilis	LMG 18870	20125	19371	27175	5512	400	2777	3	11360	2552	-135	31304
B. stabilis	LMG 18888	19737	18541	25002	1429	406	2686	12	7820	2836	-3199	8891
B. vietnamiensis	LMG 10929	1410	22218	28630	3464	528	4127	3	3693	122	-2576	9202
B. vietnamiensis	LMG 16232	7908	12968	18843	2551	263	4004	-3	2334	61	-4972	2750
B. vietnamiensis	LMG 18835	9440	13096	24187	2805	311	4013	19	1968	70	-336	1602
B. vietnamiensis	LMG 18836	824	18166	26332	1400	498	2798	9	2551	159	-2848	3766
A. baumannii	ATCC 19606	2234	1761	1948	3992	1486	8362	16	507	74	1023	55
A. calcoaceticus	7844	2286	788	1139	4352	1962	8667	9	617	58	1569	122
A. haemolyticus	12155	8775	6852	21501	8280	2472	13667	-3	1092	70	1243	-6
A. johnsonii	10308	2015	6409	13761	2875	1242	7319	7	428	61	1724	-55
A. lwoffi	5866	2326	17885	23949	1484	760	2811	6	552	49	1907	-37
A. lwoffi	5867	8277	9364	26336	3549	1425	5887	-3	787	8354	11402	88
A. lwoffi	NCIMB 12456	7609	2106	9995	592	165	12662	12	927	125	-49	289
B diminuta	ATCC 11568	4997	641	3351	23501	1856	8723	-13	662	1126	985	-9
B. vesicularis	ATCC 11426	10795	8964	14897	38775	35630	2881	0	586	6226	-61	360
R. pickettii	11149	4422	11881	20259	6440	1492	14979	0	2204	540	3397	339
C. meningosepticum	ATCC 13253	3729	650	4618	34799	35599	16099	-4	402	92	3938	638
M. nonliquefaciens	10464	9751	17680	26757	2906	528	33957	7	3580	42225	18483	-18
M. osloensis	10465	3885	247	485	1465	782	4272	-19	824	21270	19685	21
M. urethralis	11010	2240	128	306	5875	4480	10721	-19	763	16490	18666	67
O. urethralis	11999	13020	11243	15886	9171	9207	13539	6	494	33187	18923	299
P. acidovorans	10683	2442	324	2182	782	512	4895	-13	571	86	12196	-3
P. aeruginosa	6749	3284	18532	25887	1273	598	34326	-6	6245	37652	18431	-21
P. aeruginosa	10332	11945	16938	25927	5378	980	35425	18	2145	34347	18025	0
P. alcaligenes	10367	10050	1141	3766	4185	4398	30541	16	1446	8097	14683	-13
P. pseudoalcaligenes	10860	16957	12345	15739	1990	345	2075	28	1205	58	864	0
P. diminuta	8545	6202	2393	3369	37061	580	3708	-3	458	3406	363	-15
P. fluorescens	10754	16435	5060	13148	27053	35290	26516	-6	1761	32110	21403	67
P. fluorescens	10392	4703	1034	3955	7142	4169	18901	-52	1175	28265	12434	0
P. fluorescens	3756	2646	317	2042	2924	913	13032	-6	967	42285	13884	-15
P. fluorescens	10038	5775	449	2271	5442	2740	977	0	1209	44034	17827	0
P. fluorescens	10688	15077	1648	5206	8084	412	2149	-9	1037	43375	717	-9
P. fluorescens	9428	1871	16160	22173	9962	812	4062	0	885	40925	559	-25
P. fragi	NCIMB 8987	3222	186	263	1584	1214	4856	-9	790	1908	1264	613
P. maltophilia	10257	4019	15428	18590	205	4734	3782	0	1166	41498	-525	430
P. paucimobilis	11030	748	1728	6046	2985	34356	11256	-3	1303	165	-388	6635
R. pickettii	11149	1001	14329	21938	2689	1108	8244	3	1239	766	2954	-15
P. putida	10936	5377	271	1111	7626	598	3085	-25	909	36282	1355	-18
P. stutzeri	12262	13374	2826	14192	12464	58	8683	-31	964	8274	522	-24
P. stutzeri	10475	7435	1193	5982	8594	74	11301	0	1252	52	278	-21
P. vesiculare	10900	4792	406	2078	37543	34982	3015	-37	338	7893	574	333
S. spiritivorum	ATCC 33861	3525	943	7590	1963	18580	7129	12	446	1392	528	3314
B. ambifaria	11351	4859	16648	24578	15590	958	21199	-13	1761	1837	-123	3757
B. andropogonis	1279	3333	241	495	2808	1077	5201	-6	927	34122	854	174
B. andropogonis	2126	3650	293	668	7734	815	5191	-12	839	3147	1242	1489
B. caryphylli	2155	3494	9827	17784	8838	2082	28157	6	1669	25475	21462	12
B. caryphylli	2156	13914	12928	23219	24351	4605	20057	3	1651	29787	18922	2558
B. dolosa	18941	1758	14689	25762	3324	1056	34588	12	2112	19985	19603	775

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference										
Strain	**Reference**	40	41	42	43	44	45	46	47	48	49	50
B. dolosa	18942	2017	16221	25543	5457	504	2985	-6	3302	5161	3068	897
B. gladioli	11626	2359	13551	18229	14211	1056	6751	22	1346	1938	1935	79
B. gladioli	18113	1715	17491	23821	10124	1513	8534	15	4746	17723	3559	1273
B.gladioli pv. alliicola	2121	1856	15459	25313	31850	3046	11161	-21	4355	2542	1172	885
B. gladioli pv. alliicola	6877	1669	15125	23845	29961	1416	24022	3	3318	2622	1288	213
B.gladioli pv. gladioli	2216	2588	15806	23305	22816	1371	10407	7	3660	5766	809	2286
B.gladioli pv. gladioli	6880	2268	16310	24392	23106	10237	14195	3	9513	10878	6510	2149
B. glumae	1277	3573	21626	28170	17689	2143	3580	25	15895	28485	147	7258
B. glumae	2196	2331	21306	27577	20356	4920	6928	-82	16294	19204	232	125
B. phenazinium	2247	3143	4437	14760	4594	772	12770	-40	2384	25765	9235	278
B. phenazinium	6868	226	11228	20067	24144	6018	17054	13	1331	943	717	42
P. apista	16408	1935	296	534	1084	495	5335	13	790	4956	-1996	28
P. norimberensis	13019	2521	373	775	1062	494	2747	-3	512	744	1425	455
P. norimberensis	16603	2289	262	657	1560	735	3070	7	692	1190	-773	-18
P. pnomenusa	18087	1911	312	721	4267	1340	5438	27	711	79	-793	-27
P. pnomenusa	18817	2710	214	406	3565	1596	6797	7	711	235	-213	-12
P. pulmonicola	18107	1898	171	281	2021	921	4181	6	637	73	-7679	-43
P. sputorum	18100	2427	363	1044	2613	1700	4175	6	641	40	-1052	339
P. sputorum	18819	2707	302	562	5469	2267	7090	-6	833	186	-360	-9
R. basilensis	18990	3091	306	577	2832	1425	11222	0	918	97	5573	-15
R. basilensis	19286	3064	241	412	4599	2259	8005	0	1724	61	4301	-55
R. campinensis	19282	2530	171	378	1608	579	14665	-9	800	67	8481	-24
R. campinensis	19283	1654	156	491	3001	950	21004	13	909	7581	6947	229
R. eutropha	1190	1914	489	8194	2188	299	13063	15	781	19493	18321	1200
R. eutropha	1194	651	144	1935	2787	354	12114	6	827	73	5567	-46
R. gilardii	3399	2615	211	452	11771	1746	9242	3	769	67	-729	-3
R. gilardii	3400	2616	205	506	5417	1214	6543	-6	821	79	-989	24
R. mannitolilytica	19090	2631	14256	16752	12498	1023	12059	30	2451	58	1517	9
R. metallidurans	1195	6955	5844	12284	2350	837	10526	0	528	58	9248	-12
R. metallidurans	19290	2777	6842	12559	1398	531	16002	-7	769	77	8982	-52
R. paucula	3244	3183	4422	7865	4086	519	18281	6	763	68	9873	-31
R. paucula	3245	3376	4065	6821	2137	1535	17720	19	656	37	6474	-46
R. pickettii	5942	3195	11692	18245	6254	1349	12672	12	2399	67	2735	-21
R. pickettii	6871	2408	14470	20864	3253	650	7080	-3	1675	70	278	6
R. solanacearum	2291	9733	14980	21281	400	934	3839	25	872	49	1490	-22
R. solanacearum	2293	3138	376	2759	998	1016	3656	6	702	64	24	-22
R. taiwanensis	19425	2265	210	626	1093	503	2716	27	766	122	372	-24
S. maltophilia	957	2948	980	4456	257	18122	3613	18	418	42472	549	1093
S. maltophilia	958	2466	9452	13484	177	4886	2524	9	1388	43488	198	1416
H_2O		2512	95	174	18	-34	497	-25	82	58	287	-9
H_2O		2313	83	162	37	-24	543	6	85	43	843	-9
H_2O		2188	64	134	24	342	506	-3	100	37	870	-21
H_2O		2259	77	150	61	299	479	-12	85	46	616	-37
H_2O		3138	89	159	33	158	540	-12	73	49	574	-37
E. coli		2438	177	479	3559	1504	3326	6	659	25951	-802	-3
Enterobacter cloacae		2091	293	561	1599	815	2337	0	735	12556	1400	39356

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	51	52	53	54	55	56	57	58	59	60
P. aeruginosa	2688	2149	12351	302	879	574	4990	8192	40329	17912	18968
P. aeruginosa	2702	1632	10685	208	1941	1288	16487	8720	39661	18565	20076
P. aeruginosa	2704	1382	9867	201	1062	498	13743	6393	29247	19319	6086
P. aeruginosa	2706	1617	6418	464	4252	1065	2585	7655	40152	18721	19555
P. aeruginosa	2715	1877	7435	589	1581	647	2466	12919	36783	18684	3040
P. aeruginosa	2720	4175	1413	269	1101	818	5231	7804	40473	18510	-1663
P. aeruginosa	2737	1536	11409	1859	1831	1300	2747	10828	38339	19224	-4480
P. aeruginosa	2739	1459	10505	317	1200	431	1676	11320	36672	18605	-3666
P. aeruginosa	2741	1565	8381	141	1224	577	6684	7825	31469	18226	18309
P. aeruginosa	2742	1379	9900	558	1066	1249	1959	9583	37045	18058	19854
P. aeruginosa	2749	2451	8009	488	1105	565	2683	8460	39197	17928	21614
P. aeruginosa	2772	1767	10315	287	1190	565	1538	12034	35794	16874	19560
P. aeruginosa	2775	1328	77	183	1416	522	98	12578	22042	18865	19117
P. aeruginosa	2776	2329	4969	326	2362	1245	1767	12828	36453	19957	21624
P. aeruginosa	2778	1209	5619	498	1303	391	2018	9108	29443	19999	18702
P. aeruginosa	2779	2530	4498	2140	1221	467	1642	9763	40689	19426	20717
P. aeruginosa	2780	1486	9623	205	904	263	3660	9077	40524	19841	22621
P. aeruginosa	2781	1618	6214	204	1194	687	3934	9113	38056	20082	20656
P. aeruginosa	2782	3006	5167	122	1383	888	3876	10725	40076	20009	21697
P. aeruginosa	2783	1248	8695	275	1187	528	2604	8058	39362	20146	19929
P. aeruginosa	PS1	2106	12906	2427	882	232	8195	6577	40573	20110	20320
P. aeruginosa	PS2	1596	13017	149	1123	549	8851	9339	40607	20357	21184
P. aeruginosa	PS3	1416	7914	226	1538	910	5180	7484	35763	19374	19825
P. aeruginosa	PS4	1608	11689	311	1434	931	9199	7136	40173	18389	19716
P. aeruginosa	PS5	1963	8045	339	5695	1019	2265	9285	39978	18621	21368
P. aeruginosa	PS6	2200	12370	199	1370	741	10056	6964	38578	18553	20979
P. aeruginosa	PS7	2405	980	338	872	436	2622	8305	30789	18492	22613
P. aeruginosa	PS8	1248	9031	110	1294	592	3949	8103	36591	18278	22441
P. aeruginosa	PS9	2051	16588	345	1407	757	7508	8155	39615	17958	20128
P. aeruginosa	PS10	1365	11564	354	845	394	4941	7154	40421	18233	21557
P. aeruginosa	PS11	3437	9028	873	1138	479	2457	8194	37640	18779	20647
P. aeruginosa	PS12	1819	10132	186	1437	754	7175	7145	39267	18855	19072
P. aeruginosa	PS13	1215	7181	131	1132	482	2442	8970	31991	20247	19078
P. aeruginosa	PS14	1880	10657	204	928	274	1810	9327	38751	19414	19918
P. aeruginosa	PS15	1325	5475	94	2198	986	1282	8061	32836	19625	19597
P. aeruginosa	PS16	1624	11946	373	925	333	2482	8192	39868	18639	20567
P. aeruginosa	PS17	1382	11527	348	1578	574	2692	9962	38443	19097	20427
P. aeruginosa	PS18	2289	15709	367	940	440	8158	7596	40137	19029	20607
P. aeruginosa	PS19	1459	10673	329	1153	427	3858	8985	36337	19228	19609
P. aeruginosa	PS20	1380	6187	119	1392	567	1963	10962	28567	17887	20451
P. aeruginosa	PS21	1450	9379	418	995	497	4902	12037	38260	18061	18849
P. aeruginosa	PS22	1571	15248	107	1074	604	7392	6675	39506	18739	21074
P. aeruginosa	PS23	946	5656	55	1324	543	1508	9589	19728	19017	19994
P. aeruginosa	PS24	1194	275	137	513	211	339	8173	12178	18800	11698
P. aeruginosa	PS25	1422	8747	226	1541	647	1648	9195	39416	20119	20341
P. aeruginosa	PS26	2054	13294	409	962	515	6580	7905	40561	18443	21156
P. aeruginosa	PS27	1865	13383	650	1459	983	2560	10120	37015	20034	18715
P. aeruginosa	PS28	1746	13627	391	1377	998	2752	7584	40461	18785	20989

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	51	52	53	54	55	56	57	58	59	60
P. aeruginosa	PS29	1126	7874	15	772	531	3431	8573	37879	17519	20390
P. aeruginosa	PS30	1566	1856	144	1138	635	1059	8439	35608	16874	19457
P. aeruginosa	PS31	1031	9489	-6	1486	1013	3428	6419	33304	17314	20534
P. aeruginosa	PS32	1087	8655	128	824	433	3284	8015	36910	17100	19081
P. aeruginosa	PS33	2054	21325	2646	895	370	8854	6570	39664	17988	20958
P. aeruginosa	PS34	2133	11674	193	1285	726	2375	9965	34680	17747	4914
P. aeruginosa	PS35	1666	14472	208	1767	1065	1865	7248	36798	19121	21004
P. aeruginosa	PS36	1319	9293	52	879	516	4349	6421	36743	17015	21464
P. aeruginosa	PS37	2152	13154	782	1706	870	2344	11320	36285	19075	20063
P. aeruginosa	PS38	1291	13649	193	473	244	4358	6385	38849	18520	21596
P. aeruginosa	PS39	1364	13649	174	812	455	4212	7853	39536	19688	21432
P. aeruginosa	PS40	879	10713	177	919	647	7655	7444	39890	18684	20866
P. aeruginosa	PS41	1703	18187	336	1123	712	4807	6781	40131	17527	19450
P. aeruginosa	PS42	1078	12562	34	1416	1102	4206	7227	40201	18346	21739
P. aeruginosa	PS43	2085	14308	290	787	525	4279	8622	40167	17064	19081
P. aeruginosa	PS44	3567	7246	565	1282	650	1157	10902	36224	16871	8479
P. aeruginosa	PS45	3754	11454	1129	964	488	1651	10324	37763	17384	21220
P. aeruginosa	PS46	1004	8512	296	552	269	3095	8179	39923	16877	20171
P. aeruginosa	PS47	1346	10557	104	799	342	2079	8091	35342	17195	19252
P. aeruginosa	PS48	3449	16200	1090	1047	616	3946	7657	40604	17985	21782
P. aeruginosa	PS49	3512	8430	440	1098	641	2427	6607	33541	18483	21013
P. aeruginosa	PS50	1398	4297	180	1386	726	950	6824	38455	17857	19353
P. aeruginosa	PS51	1550	15618	202	955	601	8637	8802	40183	17772	20198
P. aeruginosa	PS52	1587	16511	4843	756	351	2494	6931	39966	17705	19566
B. cepacia	LMG 1222	1007	32696	15669	1087	367	5029	5985	40655	20503	-4947
B. cepacia	LMG 2161	1092	17247	17414	970	202	4422	8280	40537	17797	-5161
B. cenocepacia	LMG 16654	891	28271	8122	574	158	6904	6559	40741	19838	-5506
B. cenocepacia	LMG 16656	1307	36099	16252	653	217	13219	6285	40643	19865	-5085
B. cenocepacia	LMG 16659	1541	21800	34955	540	125	4273	7050	40192	18409	-5359
B. cepacia	LMG 17997	864	26736	5616	1545	354	3665	6165	40253	19148	-5115
B. cepacia	LMG 18821	546	25878	9650	1138	305	1712	6339	40183	18352	-4759
B. cenocepacia	LMG 18826	2930	27431	24999	1242	412	1252	5982	40247	18388	-4822
B. cenocepacia	LMG 18827	1547	19972	10230	943	320	2509	6040	40680	18010	-4712
B. cenocepacia	LMG 18828	1020	32892	11989	564	202	8219	6360	40063	19365	-4654
B. cenocepacia	LMG 18829	1175	29296	15498	870	391	8753	6208	39832	19508	-4633
B. cenocepacia	LMG 18830	1047	31189	12913	556	195	9211	6074	39380	18785	-3916
B. cenocepacia	LMG 18832	1657	20345	34488	3699	638	3538	7654	40762	17274	-4562
B. cenocepacia	LMG 18863	1554	24138	24919	2081	473	12321	6501	40961	18077	-4639
B. multivorans	LMG 13010	1117	32848	21208	1117	297	7173	6201	41284	20567	-4673
B. multivorans	LMG 16660	1166	31933	14222	1111	351	5097	6186	40649	20018	-4804
B. multivorans	LMG 16665	1129	32311	8924	602	339	9043	6134	40415	20613	-4676
B. multivorans	LMG 17588	1703	27636	15160	900	479	5421	5949	40626	17879	-4484
B. multivorans	LMG 18822	2469	25255	21282	1095	464	5237	6080	40570	17729	-4517
B. multivorans	LMG 18823	2744	30309	24068	619	263	4389	5942	40546	17876	-4410
B. multivorans	LMG 18824	1441	22216	15892	580	293	3580	7044	40115	17186	-4846
B. multivorans	LMG 18825	2087	25771	25072	1099	677	1588	6122	39634	17424	-3833
B. stabilis	LMG 14086	1022	26296	18226	635	211	5973	6486	39731	17827	-3699
B. stabilis	LMG 14294	500	25442	13072	748	278	6617	5857	39185	16722	-3232

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	51	52	53	54	55	56	57	58	59	60
B. stabilis	LMG 18870	775	30645	14952	635	589	9757	6892	39271	16615	-601
B. stabilis	LMG 18888	-110	33096	18959	1245	751	12498	6678	39618	16990	-4089
B. vietnamiensis	LMG 10929	494	27673	24169	1532	952	14622	7173	40042	17638	-2090
B. vietnamiensis	LMG 16232	1581	19197	16639	900	754	1437	6983	38351	16728	-2897
B. vietnamiensis	LMG 18835	1025	17439	14635	784	659	1331	7056	36294	16585	-2246
B. vietnamiensis	LMG 18836	522	27883	20238	1870	751	10480	7260	39563	16863	-2255
A. baumannii	ATCC 19606	1709	568	522	1175	2103	274	8302	4114	16914	-833
A. calcoaceticus	7844	2570	272	604	2002	4606	204	8351	1691	16978	-497
A. haemolyticus	12155	1703	378	452	1859	4017	351	9065	37268	16441	-1090
A. johnsonii	10308	837	89	165	1691	2851	73	7133	4794	16689	-443
A. lwoffi	5866	482	92	152	885	1139	94	6699	5155	16347	-632
A. lwoffi	5867	1792	9955	715	1566	2802	2298	7462	37906	15266	4441
A. lwoffi	NCIMB 12456	454	140	897	842	882	119	6995	4386	16755	-989
B diminuta	ATCC 11568	1706	161	13661	2389	4786	232	7991	6894	20994	-1837
B. vesicularis	ATCC 11426	9050	226	35507	1532	42768	119	8735	10352	26110	-2259
R. pickettii	11149	6482	25258	781	2042	2847	1611	7434	35574	20955	-784
C. meningosepticum	ATCC 13253	33807	425	24367	35364	42914	180	35055	9235	29241	2170
M. nonliquefaciens	10464	2323	22402	766	1654	1026	7590	11347	40353	19277	22282
M. osloensis	10465	19258	13914	2158	1349	650	2509	15941	36267	24211	25637
M. urethralis	11010	13691	18172	565	973	638	85	18077	3278	22487	-1215
O. urethralis	11999	30703	22820	14805	14818	23443	821	25802	40042	26424	24288
P. acidovorans	10683	1736	171	1169	1309	830	137	7078	5979	17384	7593
P. aeruginosa	6749	6824	22768	354	1291	1288	10148	8228	38806	15858	20781
P. aeruginosa	10332	1557	20799	531	1539	1160	5280	7991	39053	16582	21960
P. alcaligenes	10367	208	4600	198	654	528	2701	6812	35031	16875	19945
P. pseudoalcaligenes	10860	818	126	537	2042	1007	519	15511	21162	22692	-1978
P. diminuta	8545	1715	232	24282	1828	882	137	7102	22463	27831	21498
P. fluorescens	10754	12315	7630	3674	13856	24752	8219	25515	36767	27089	23158
P. fluorescens	10392	955	2949	167	1966	1755	162	11329	6345	17329	14368
P. fluorescens	3756	3485	299	333	2689	2353	329	11677	3375	17818	18150
P. fluorescens	10038	4440	244	18083	3397	4102	110	8476	4215	17241	20763
P. fluorescens	10688	1044	165	1077	1984	2161	40	10270	2780	17359	-1996
P. fluorescens	9428	724	244	186	1346	1501	83	8900	5097	17415	-2268
P. fragi	NCIMB 8987	1526	79	9214	1358	1910	79	7630	729	16823	-24
P. maltophilia	10257	2735	1825	25628	5814	6681	863	6944	19399	27755	-974
P. paucimobilis	11030	30941	607	16190	33951	40473	662	18987	12117	26577	-1312
R. pickettii	11149	3837	20918	305	1984	1407	6357	7328	41513	17610	-34
P. putida	10936	1892	110	562	2658	1089	122	10782	7163	17408	-2005
P. stutzeri	12262	1050	760	143	2826	2378	467	21196	26595	17564	-1907
P. stutzeri	10475	1226	589	168	2332	1740	213	26199	23849	24770	-1099
P. vesiculare	10900	2548	226	21508	2634	37625	149	10172	38428	29516	24617
S. spiritivorum	ATCC 33861	12208	180	3620	6943	19695	204	28408	9839	24865	-1599
B. ambifaria	11351	503	13585	4420	1728	1865	2512	7029	42002	20681	-2597
B. andropogonis	1279	2564	1117	22887	5859	5804	2686	7419	22622	17558	-1953
B. andropogonis	2126	15883	174	10520	7197	15568	189	11246	16419	20220	-1718
B. caryphylli	2155	15611	19478	2148	5289	4810	2054	12037	38601	20217	-351
B. caryphylli	2156	15068	22231	5173	1334	1870	73	10523	39685	19743	-1132
B. dolosa	18941	1892	8076	11799	1410	1596	1309	7383	31136	17396	23305

Appendix 3.2a (cont.): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference									
Strain	**Reference**	51	52	53	54	55	56	57	58	59	60
B. dolosa	18942	6293	4044	12571	2530	1218	2106	12367	33379	20708	2252
B. gladioli	11626	1142	15474	1449	1581	2185	1953	7282	38873	17558	-330
B. gladioli	18113	2570	25273	5261	1569	1776	8158	9089	41474	18660	1111
B.gladioli pv. alliicola	2121	1392	16771	2481	2270	1764	7022	8613	41388	17747	-543
B. gladioli pv. alliicola	6877	2179	17262	2194	1294	1550	6757	8735	41428	17451	247
B.gladioli pv. gladioli	2216	3656	23565	4474	1657	1774	10563	10331	41358	18632	-1279
B.gladioli pv. gladioli	6880	3663	27642	5271	2063	1916	13026	7710	40140	17589	3696
B. glumae	1277	1535	26265	2859	3980	5976	16286	10658	40116	17525	-2335
B. glumae	2196	1377	23363	2506	3656	4005	11100	7929	39823	17622	-2115
B. phenazinium	2247	6858	4691	7575	8851	14897	2322	29876	10514	28158	1846
B. phenazinium	6868	1273	1657	6211	5484	15178	5017	7661	37995	17021	21279
P. apista	16408	6645	2673	3129	8216	19157	2573	17961	29489	23931	22436
P. norimberensis	13019	30138	662	13081	22487	35260	341	9218	2393	19973	-3080
P. norimberensis	16603	962	70	1016	3696	2603	110	6937	790	17222	-3983
P. pnomenusa	18087	2121	71	207	1438	1071	113	7279	4871	17314	-2918
P. pnomenusa	18817	1978	1307	1028	1272	1199	113	9064	27938	19096	-2066
P. pulmonicola	18107	-412	30	9434	290	828	287	6498	17146	17228	-3608
P. sputorum	18100	36	159	137	15593	23873	7999	18660	36422	22585	4029
P. sputorum	18819	766	67	100	2493	1965	122	7068	1309	17335	-2692
R. basilensis	18990	2454	94	6620	876	869	485	7056	19151	18254	3086
R. basilensis	19286	1895	95	7859	1419	1428	94	10621	8637	22466	1218
R. campinensis	19282	2890	74	1343	1056	1175	98	7971	1276	21565	7407
R. campinensis	19283	13944	4502	7459	9037	21837	4794	12898	36920	20543	23348
R. eutropha	1190	8557	10893	12492	7813	18911	4346	7609	22105	17106	8298
R. eutropha	1194	2881	885	11662	2655	1089	204	7333	24874	17127	3628
R. gilardii	3399	2979	40	4923	1517	1181	88	7343	619	17176	-2512
R. gilardii	3400	2622	22	7197	1538	1291	67	7273	528	17284	-2232
R. mannitolilytica	19090	11759	5055	5396	30785	43036	787	20696	33804	23900	-766
R. metallidurans	1195	2005	732	2667	2707	1410	204	7053	31267	17362	5064
R. metallidurans	19290	1496	476	629	1221	1144	150	7437	34570	17536	8024
R. paucula	3244	2710	19240	134	2023	1382	6550	7831	38940	17048	8186
R. paucula	3245	805	3229	119	2686	4025	745	7425	33227	17382	3748
R. pickettii	5942	3150	18731	161	1710	1206	4123	7642	38858	17070	1242
R. pickettii	6871	1956	21688	421	1547	1176	3492	7545	36765	16283	-833
R. solanacearum	2291	2393	183	656	1398	2344	3989	14125	36297	21251	19444
R. solanacearum	2293	1419	31	516	1513	1145	116	9947	34145	19023	10651
R. taiwanensis	19425	12406	67	6180	12669	14594	4221	19481	39325	22811	8741
S. maltophilia	957	17256	223	32739	8488	11079	268	7465	10148	26952	-1239
S. maltophilia	958	8989	2343	38678	8094	9049	1010	7328	21846	30230	-1401
H_2O		245	31	107	577	525	79	6992	2057	16944	-1022
H_2O		201	43	61	546	541	79	7020	2417	17128	-879
H_2O		321	22	64	473	598	55	6946	2621	17037	-1108
H_2O		229	28	73	537	553	55	7065	2167	16911	-1020
H_2O		241	31	67	476	522	76	6916	2374	16887	-760
E. coli	10418	1648	113	7499	1391	1227	94	11427	1104	15949	-2524
Enterobacter cloacae	11936	1321	80	1669	1779	2069	97	7493	913	15724	226

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	61	62	63	64	65	66	67	68	69
P. aeruginosa	2688	-150	-12	-31	-28	-43	-128	-2296	903	43
P. aeruginosa	2702	-122	-6	-31	-55	-46	287	2424	973	37
P. aeruginosa	2704	-171	-3	-25	-37	-52	80	-382	958	52
P. aeruginosa	2706	-153	13	-49	-43	-58	94	-3016	1111	49
P. aeruginosa	2715	-125	-40	-34	-61	-52	-141	-4078	852	70
P. aeruginosa	2720	-149	3	-40	-49	-61	-226	-6345	870	49
P. aeruginosa	2737	-122	219	320	-9	-15	-79	-4853	1550	98
P. aeruginosa	2739	-116	-6	-43	-55	-28	-77	-7688	1016	67
P. aeruginosa	2741	-146	-21	-55	-52	-31	-159	-6855	733	43
P. aeruginosa	2742	-131	162	-40	-12	-49	-143	-6706	736	83
P. aeruginosa	2749	-146	40	-46	-43	-52	165	-6562	952	37
P. aeruginosa	2772	-128	37	-31	55	-65	73	-5521	982	43
P. aeruginosa	2775	-131	129	3	31	-58	98	-5552	962	107
P. aeruginosa	2776	-131	77	-52	12	-45	76	-6498	1099	113
P. aeruginosa	2778	-152	-3	-21	-28	-42	-226	-7645	940	52
P. aeruginosa	2779	-153	22	-31	15	-39	-43	-7593	1059	92
P. aeruginosa	2780	2274	28	6497	1861	-52	-213	-6674	1001	52
P. aeruginosa	2781	-107	-6	-16	-15	-52	-193	-6855	1047	46
P. aeruginosa	2782	-149	-9	-12	-19	-43	-123	-3605	900	9
P. aeruginosa	2783	-153	52	-40	-3	-58	-28	-6955	1193	28
P. aeruginosa	PS1	-138	24	-43	-31	-31	-104	-7141	864	37
P. aeruginosa	PS2	-126	-9	-37	-21	-54	-125	-3745	912	37
P. aeruginosa	PS3	-125	18	-43	-46	-52	-143	-3696	1266	37
P. aeruginosa	PS4	-135	-27	-28	-46	-45	-211	-5939	1255	34
P. aeruginosa	PS5	-149	12	-34	-9	-54	-159	-3830	949	40
P. aeruginosa	PS6	-137	49	-40	-25	-55	-125	-6324	1242	40
P. aeruginosa	PS7	-161	162	332	-52	-30	-202	-6830	1346	64
P. aeruginosa	PS8	-116	52	-28	-46	-27	-168	-6317	1318	43
P. aeruginosa	PS9	-129	46	-49	-31	-61	-165	-5393	1618	0
P. aeruginosa	PS10	-140	28	-58	-55	-67	-187	-6986	1001	43
P. aeruginosa	PS11	-153	24	-18	-31	-49	-138	-3659	1291	46
P. aeruginosa	PS12	-155	0	-22	-58	-42	-211	-6419	1361	52
P. aeruginosa	PS13	-134	650	1092	-15	-55	-174	-7160	934	55
P. aeruginosa	PS14	-147	3	-43	-15	-45	-113	-7160	1077	61
P. aeruginosa	PS15	-140	608	1315	15	-58	-125	-4862	986	40
P. aeruginosa	PS16	-162	-9	189	-24	-46	-190	-4444	1050	43
P. aeruginosa	PS17	-164	43	-43	-58	-55	-162	-7114	983	37
P. aeruginosa	PS18	-165	3	-40	-46	-55	-199	-5328	1553	28
P. aeruginosa	PS19	-159	67	-40	21	-51	-43	-6870	965	70
P. aeruginosa	PS20	-150	836	2344	-6	-55	-164	-8060	1034	64
P. aeruginosa	PS21	-153	6	-25	-34	-52	-195	-7074	1172	94
P. aeruginosa	PS22	-134	12	-46	-3	-34	-216	-6083	1606	37
P. aeruginosa	PS23	-152	497	851	-18	-49	-186	-7163	1056	76
P. aeruginosa	PS24	-155	21	-49	-27	-55	-223	-7310	970	58
P. aeruginosa	PS25	-171	58	-34	-46	-49	-134	-4480	940	68
P. aeruginosa	PS26	-156	18	-40	-67	-34	-189	-7034	1438	40
P. aeruginosa	PS27	-149	40	-37	9	-52	-196	-6907	876	25
P. aeruginosa	PS28	-159	-24	-52	-46	-55	-223	-2536	1321	12

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	61	62	63	64	65	66	67	68	69
P. aeruginosa	PS29	-146	515	744	-3	-48	-116	-4032	1044	52
P. aeruginosa	PS30	-146	104	-25	-12	-52	-40	-6971	946	40
P. aeruginosa	PS31	-152	717	1327	-37	-49	-204	-7307	876	40
P. aeruginosa	PS32	-183	70	-28	-19	-46	-198	-7556	925	64
P. aeruginosa	PS33	-137	61	-37	-37	-40	-146	-7373	950	43
P. aeruginosa	PS34	-134	24	-67	-13	-58	-186	-6384	1004	67
P. aeruginosa	PS35	-100	37	-52	-49	-70	-198	-7484	821	40
P. aeruginosa	PS36	-119	39	-46	-37	-45	-214	-6943	1578	52
P. aeruginosa	PS37	-153	40	-46	-28	-52	-171	-6227	937	58
P. aeruginosa	PS38	-143	674	1276	-40	-55	-187	-6467	818	52
P. aeruginosa	PS39	-131	89	1266	-3	-58	-156	-5726	876	52
P. aeruginosa	PS40	-177	802	1608	-9	-51	-190	-6861	772	31
P. aeruginosa	PS41	-141	107	0	9	-55	-39	-1612	824	36
P. aeruginosa	PS42	-128	1114	2481	-9	-48	-202	-6937	977	25
P. aeruginosa	PS43	-144	71	-34	33	-55	-110	-4020	952	37
P. aeruginosa	PS44	-131	452	738	40	-82	-122	-6852	1263	85
P. aeruginosa	PS45	-137	156	-22	24	-18	-55	-4762	1339	61
P. aeruginosa	PS46	-158	64	-22	37	-30	-199	-5277	812	64
P. aeruginosa	PS47	-174	164	214	-31	-15	-239	-6800	922	73
P. aeruginosa	PS48	-146	64	-43	-3	-21	-244	-4182	1745	70
P. aeruginosa	PS49	-146	28	-55	-34	-65	-210	-5542	1108	52
P. aeruginosa	PS50	-165	45	-28	-12	-52	-189	-4768	906	70
P. aeruginosa	PS51	-150	39	-9	9	-46	-217	-4523	873	13
P. aeruginosa	PS52	-147	30	-18	18	-52	-183	-6809	796	43
B. cepacia	LMG 1222	17079	2063	39645	39752	-27	-192	2451	857	138
B. cepacia	LMG 2161	424	1154	25884	38726	38794	-168	2942	854	40
B. cenocepacia	LMG 16654	7988	1813	29259	40335	-33	-226	1535	867	83
B. cenocepacia	LMG 16656	3009	1804	23363	40320	-27	-190	1962	845	79
B. cenocepacia	LMG 16659	483	635	17570	37539	38004	-198	2064	808	22
B. cepacia	LMG 17997	3403	1300	25918	40057	-42	-192	-18	821	52
B. cepacia	LMG 18821	67	608	19490	39831	17159	-183	-5213	775	110
B. cenocepacia	LMG 18826	159	644	21745	39007	24920	-211	-5020	714	131
B. cenocepacia	LMG 18827	-159	286	204	12	-33	-183	-7135	778	40
B. cenocepacia	LMG 18828	6094	2097	33093	40128	-42	-217	635	760	86
B. cenocepacia	LMG 18829	4475	1969	33941	40009	-48	-213	1493	799	82
B. cenocepacia	LMG 18830	7468	2615	39310	39670	-49	-204	3076	720	52
B. cenocepacia	LMG 18832	86	708	5737	37384	-49	-189	-3064	976	58
B. cenocepacia	LMG 18863	-43	736	4789	38074	-48	-189	-107	848	37
B. multivorans	LMG 13010	13651	2497	35522	39755	-33	-122	1889	839	100
B. multivorans	LMG 16660	3894	1825	30923	39780	-55	-156	2173	781	162
B. multivorans	LMG 16665	7142	2411	36319	39533	-34	-159	2298	821	98
B. multivorans	LMG 17588	-147	275	204	33	-40	-125	-7072	989	43
B. multivorans	LMG 18822	-159	241	168	-9	-49	-134	-6751	1264	34
B. multivorans	LMG 18823	-140	290	244	55	-43	-131	-7505	757	40
B. multivorans	LMG 18824	-159	216	43	6	-79	-171	-7087	724	55
B. multivorans	LMG 18825	-165	232	27	18	-71	-40	-6840	656	43
B. stabilis	LMG 14086	-144	131	851	37878	31695	-248	1394	784	485
B. stabilis	LMG 14294	-104	134	1419	37692	27242	-189	339	699	391

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	61	62	63	64	65	66	67	68	69
B. stabilis	LMG 18870	21397	3848	37518	37750	52	52	22756	1019	82
B. stabilis	LMG 18888	55	116	1053	36447	39564	34	26086	870	540
B. vietnamiensis	LMG 10929	3	666	296	1224	24	-12	5274	916	30
B. vietnamiensis	LMG 16232	-6	19	189	104	3	128	5421	900	16
B. vietnamiensis	LMG 18835	-22	24	150	73	9	18	5503	851	12
B. vietnamiensis	LMG 18836	-18	638	186	34329	37	27	7105	922	33
A. baumannii	ATCC 19606	-31	-34	18	73	7	6	5008	2323	25
A. calcoaceticus	7844	-7	-42	-3	238	-10	7	5173	2832	211
A. haemolyticus	12155	-28	3	28	195	25	19	3007	1612	15
A. johnsonii	10308	-55	-58	15	30	-3	16	4194	1813	-9
A. lwoffi	5866	-34	-58	3	146	-10	19	4456	1603	-3
A. lwoffi	5867	-49	-97	0	64	3	3	5783	2390	6
A. lwoffi	NCIMB 12456	-24	-40	140	174	19	6	3544	2225	19
B diminuta	ATCC 11568	-37	-42	36	85	-3	28	5280	2131	183
B. vesicularis	ATCC 11426	10746	109	38546	21	16	396	4562	849	6
R. pickettii	11149	0	-40	67	217	6	9	4945	1886	25
C. meningosepticum	ATCC 13253	253	55	14171	19957	14601	507	4938	1529	15
M. nonliquefaciens	10464	6	39	12	73	12	77	24419	1072	22
M. osloensis	10465	0	76	196	58	9	40	20900	1352	9
M. urethralis	11010	-28	9	9	64	3	28	10029	922	-9
O. urethralis	11999	-16	12	323	684	327	46	16114	1285	24
P. acidovorans	10683	3	-9	18	15	-6	3	5084	918	5124
P. aeruginosa	6749	-9	-40	635	327	144	40	13075	1096	25
P. aeruginosa	10332	-6	34	46	333	37	137	24242	1431	31
P. alcaligenes	10367	-43	30	4777	574	-4	21	16688	1187	86
P. pseudoalcaligenes	10860	0	-3	89	40	16	16	3406	2029	36
P. diminuta	8545	223	22	-9	342	-7	3	5118	1175	18
P. fluorescens	10754	49	-9	5317	5906	3205	126	17549	1432	708
P. fluorescens	10392	24	27	137	226	31	177	12190	852	293
P. fluorescens	3756	-52	6	18	33	6	30	7581	970	0
P. fluorescens	10038	-24	9	0	85	-24	55	24822	864	25
P. fluorescens	10688	-34	-21	6	31	9	13	18373	1246	22
P. fluorescens	9428	0	-3	-9	70	0	-6	4678	870	202
P. fragi	NCIMB 8987	-18	116	360	504	6150	25	4874	839	22
P. maltophilia	10257	2725	3	8625	4633	290	128	4993	1062	15
P. paucimobilis	11030	20510	27810	32381	34774	5927	678	3410	1462	15
R. pickettii	11149	-3	-3	70	70	36	27	7163	1483	73
P. putida	10936	-22	-15	-9	101	9	36	27211	830	61
P. stutzeri	12262	-37	9	-27	85	-4	27	24538	864	3
P. stutzeri	10475	-34	-16	-3	119	3	9	4121	1053	0
P. vesiculare	10900	4929	94	20768	3317	100	140	5274	937	49
S. spiritivorum	ATCC 33861	4926	263	15019	15596	8634	787	5417	2353	73
B. ambifaria	11351	34	634	3110	37851	-7	15	10685	784	9
B. andropogonis	1279	-25	565	49	52	24	33	9256	930	9
B. andropogonis	2126	3314	42	9556	8439	7718	485	5210	803	43
B. caryphylli	2155	125	-70	3702	3611	3140	55	23323	1056	25
B. caryphylli	2156	7975	6660	26534	491	669	641	5130	897	74
B. dolosa	18941	-24	61	260	281	3864	40	6418	894	19

Appendix 3.2a (cont..): Increases in fluorescence caused by various bacteria due to hydrolysis of fluorogenic substrates after 18 hr incubation.

		Substrate reference								
Strain	**Reference**	61	62	63	64	65	66	67	68	69
B. dolosa	18942	607	58	1715	800	3171	174	5525	998	34
B. gladioli	11626	-28	31	21	20342	16	22	5182	861	9
B. gladioli	18113	-25	15	34	26500	3	18	4794	879	3
B.gladioli pv. alliicola	2121	-3	40	251	37033	19	12	5536	891	-9
B. gladioli pv. alliicola	6877	-16	12	25	30938	-10	27	5323	848	15
B.gladioli pv. gladioli	2216	-24	49	128	26723	10	24	5640	858	9
B.gladioli pv. gladioli	6880	-22	-19	46	35425	0	0	5399	815	12
B. glumae	1277	-6	79	125	24	524	-10	5228	782	6
B. glumae	2196	-22	24	3	37	628	18	5448	818	7
B. phenazinium	2247	-22	3	12	30	16	-6	5048	849	71
B. phenazinium	6868	-49	42	9	34	0	9	4990	864	-3
P. apista	16408	1645	104	6599	4370	5549	744	14677	1608	-3
P. norimberensis	13019	128	3	2707	2173	1574	58	4911	1407	7
P. norimberensis	16603	-28	0	-21	12	16	40	4486	790	28
P. pnomenusa	18087	24	19	9	37	-25	-24	4798	845	-30
P. pnomenusa	18817	-31	15	-6	30	4	-6	5710	864	-3
P. pulmonicola	18107	-40	27	9	21	16	-18	-1547	742	3
P. sputorum	18100	40	24	4953	4508	2006	82	18876	1221	49
P. sputorum	18819	-21	-12	6	3	10	9	4453	836	89
R. basilensis	18990	-12	-3	-15	0	22	9	5225	931	205
R. basilensis	19286	-12	-6	217	25	19	16	4980	1319	333
R. campinensis	19282	-15	-43	-3	82	18	-37	4649	1355	-9
R. campinensis	19283	3876	113	6791	3641	6696	690	15089	1459	12
R. eutropha	1190	-18	-45	-9	37	-13	0	5360	1456	64
R. eutropha	1194	-37	-9	0	82	-12	-19	5008	879	497
R. gilardii	3399	-36	-6	153	30	-6	10	5203	809	351
R. gilardii	3400	-18	12	217	28	-12	22	5402	833	525
R. mannitolilytica	19090	88	40	5228	4517	3137	143	4984	1535	52
R. metallidurans	1195	-3	9	-49	36	-28	18	4877	1383	495
R. metallidurans	19290	-30	15	0	0	-31	15	5069	1200	296
R. paucula	3244	-16	0	3	37	-31	-9	5213	1233	3
R. paucula	3245	-25	3	-12	6	-16	16	4951	1538	18
R. pickettii	5942	-37	-9	-9	79	-7	3	4945	1392	36
R. pickettii	6871	-34	0	-9	21	27	6	4639	946	55
R. solanacearum	2291	-58	-42	31	37	21	18	5118	827	58
R. solanacearum	2293	-43	-58	15	49	0	103	4971	882	131
R. taiwanensis	19425	9	-33	2069	1575	1487	58	14400	1285	21
S. maltophilia	957	842	-19	14259	3669	6312	610	4517	922	3
S. maltophilia	958	13920	-25	20689	11561	4285	747	4270	1096	0
H_2O		-34	-6	15	-21	-3	18	4813	806	-4
H_2O		-46	9	-18	-12	-10	18	4801	769	10
H_2O		-43	13	-6	3	3	3	4908	729	3
H_2O		-43	3	-6	21	-10	-9	5505	717	0
H_2O		-31	3	-12	3	-19	0	4972	779	-12
E. coli	10418	-28	174	89	67	3821	70	4511	784	9
Enterobacter cloacae	11936	122	250	1328	1944	2505	28	4215	809	18

Appendix 3.2b: Key to fluorogenic substrates

Substrate no.	Substrate
1	4-methylumbelliferyl-ß-D-galactoside
2	4-methylumbelliferyl-ß-D-glucoside
3	4-methylumbelliferyl-N-acetyl-ß-D-glucosaminide
4	4-methylumbelliferyl-ß-D-glucuronide
5	4-methylumbelliferyl-ß-D-xyloside
6	4-methylumbelliferyl-α-D-glucoside
7	4-methylumbelliferyl-α-D-galactoside
8	4-methylumbelliferyl-ß-D-fucoside
9	4-methylumbelliferyl-α-D-manoside
10	4-methylumbelliferyl phosphate
11	4-methylumbelliferyl sulphate
12	L-alanyl-7-amido-4-methylcoumarin
13	L-leucyl-7-amido-4-methylcoumarin
14	L-prolyl-7-amido-4-methylcoumarin
15	L-phenylalanyl-7-amido-4-methylcoumarin
16	L-glutamyl-7-amido-4-methylcoumarin
17	L-lysyl-7-amido-4-methylcoumarin
18	L-asparaginyl-7-amido-4-methylcoumarin
19	4-methylumbelliferyl-ß-D-cellobioside
20	glycyl-L-prolyl-7-amido-4-methylcoumarin
21	Z-arginyl-7-amido-4-methylcoumarin
22	4-methylumbelliferyl acetate
23	4-methylumbelliferyl butyrate
24	4-methylumbelliferyl-α-L-arabinoside
25	4-methylumbelliferyl-α-D-glucuronide
26	L-aspartyl-7-amido-4-methylcoumarin
27	4-methylumbelliferyl-ß-D-riboside
28	L-arginyl-7-amido-4-methylcoumarin
29	L-isoleucyl-7-amido-4-methylcoumarin
30	4-methylumbelliferyl-ß-D-mannoside
31	L-ornithyl-7-amido-4-methylcoumarin
32	L-threonyl-7-amido-4-methylcoumarin
33	4-methylumbelliferyl propionate
34	4-methylumbelliferyl heptanoate
35	4-methylumbelliferyl nonanoate
36	4-methylumbelliferyl laurate
37	4-methylumbelliferyl stearate
38	glycyl-7-amido-4-methylcoumarin
39	L-histidyl-7-amido-4-methylcoumarin
40	ß-alanyl-7-amido-4-methylcoumarin
41	4-methylumbelliferyl elaidate
42	4-methylumbelliferyl oleate
43	L-tyrosyl-7-amido-4-methylcoumarin
44	L-valyl-7-amido-4-methylcoumarin
45	L-glutamic acid-7-amido-4-methylcoumarin
46	4-methylumbelliferyl-lignocerate
47	4-methylumbelliferyl-palmitate
48	4-methylumbelliferyl-ß-D-riboside (duplicate)
49	L-pyroglutamyl-7-amido-4-methylcoumarin
50	4-methylumbelliferyl-ß-D-xyloside (duplicate)

Appendix 3.2b (cont`d..): Key to fluorogenic substrates

Substrate no.	Substrate
51	L-threonyl-7-amido-4-methylcoumarin (duplicate)
52	4-methylumbelliferyl-palmitate (duplicate)
53	4-methylumbelliferyl phosphate (duplicate)
54	L-histidyl-7-amido-4-methylcoumarin (duplicate)
55	L-valyl-7-amido-4-methylcoumarin (duplicate)
56	4-methylumbelliferyl stearate (duplicate)
57	L-ornithyl-7-amido-4-methylcoumarin (duplicate)
58	4-methylumbelliferyl oleate (duplicate)
59	glycyl-L-prolyl-7-amido-4-methylcoumarin (duplicate)
60	L-pyroglutamyl-7-amido-4-methylcoumarin (duplicate)
61	4-methylumbelliferyl-ß-D-cellobioside (duplicate)
62	4-methylumbelliferyl-ß-D-fucoside (duplicate)
63	4-methylumbelliferyl-ß-D-glucoside (duplicate)
64	4-methylumbelliferyl-N-acetyl-ß-D-glucosaminide (duplicate)
65	4-methylumbelliferyl-α-D-galactoside (duplicate)
66	4-methylumbelliferyl-α-D-glucuronide (duplicate)
67	ß-alanyl-7-amido-4-methylcoumarin (duplicate)
68	4-methylumbelliferyl-*p* -guanidinobenzoate
69	4-methylumbelliferyl-sulphate (duplicate)

Appendix 3.3a: Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**
P. aeruginosa	2688	-0.237	0.25	0.544	0.137	0.267
P. aeruginosa	2702	-0.275	0.953	0.378	0.117	0.068
P. aeruginosa	2704	-0.148	1.515	0.38	0.235	0.152
P. aeruginosa	2706	-0.222	0.41	0.512	0.346	0.226
P. aeruginosa	2715	-0.28	0.959	0.003	-0.041	1.465
P. aeruginosa	2720	-0.281	0.084	0.034	-0.038	0.37
P. aeruginosa	2737	0.198	2.176	-0.011	-0.026	1.374
P. aeruginosa	2739	-0.161	1.009	0.022	-0.293	0.081
P. aeruginosa	2741	-0.177	0.013	0.009	-0.063	0.305
P. aeruginosa	2742	0	0.791	0.249	0.105	0.392
P. aeruginosa	2749	0.213	0.76	-0.002	-0.024	1.752
P. aeruginosa	2772	-0.213	1.062	0.062	-0.031	0.501
P. aeruginosa	2775	-0.075	1.585	0.003	-0.028	0.222
P. aeruginosa	2776	0.279	1.167	0.004	-0.05	0.206
P. aeruginosa	2778	-0.12	0.193	0.03	0.18	0.375
P. aeruginosa	2779	-0.284	0.373	0.006	-0.213	0.13
P. aeruginosa	2780	-0.187	0.876	-0.003	-0.05	0.148
P. aeruginosa	2781	-0.083	0.529	0.026	-0.052	0.642
P. aeruginosa	2782	-0.036	1.221	0	-0.038	0.432
P. aeruginosa	2783	-0.143	1.094	0.174	-0.134	0.349
P. aeruginosa	PS1	-0.236	0.492	-0.009	-0.055	0.186
P. aeruginosa	PS2	-0.177	0.984	0.113	-0.011	0.944
P. aeruginosa	PS3	-0.181	0.426	0.107	0.016	0.156
P. aeruginosa	PS4	-0.218	0.944	0.123	0.219	0.131
P. aeruginosa	PS5	-0.144	1.034	0.019	-0.042	1.523
P. aeruginosa	PS6	-0.082	0.853	0.276	0.256	0.144
P. aeruginosa	PS7	-0.076	0.373	0.353	0.141	0.321
P. aeruginosa	PS8	0.148	0.127	0.11	-0.335	0.066
P. aeruginosa	PS9	-0.342	0.774	0.597	0.594	0.362
P. aeruginosa	PS10	-0.237	1.204	0.129	0.152	0.176
P. aeruginosa	PS11	-0.329	0.483	0.005	0.051	2.034
P. aeruginosa	PS12	-0.11	1.052	0.188	0.3	0.177
P. aeruginosa	PS13	-0.075	1.607	0.19	0.084	0.334
P. aeruginosa	PS14	-0.112	0.809	0.038	0.059	1.128
P. aeruginosa	PS15	-0.084	0.998	0.22	0.019	0.131
P. aeruginosa	PS16	-0.21	0.618	0.427	0.509	0.136
P. aeruginosa	PS17	-0.183	0.2	0.164	-0.03	0.153
P. aeruginosa	PS18	-0.101	1.012	0.346	0.267	0.33
P. aeruginosa	PS19	0.085	1.19	0.331	0.486	1.02
P. aeruginosa	PS20	-0.111	1.083	0.104	-0.033	0.317
P. aeruginosa	PS21	-0.061	0.917	0.126	0.067	0.128
P. aeruginosa	PS22	-0.139	0.622	0.213	0.122	0.162
P. aeruginosa	PS23	-0.05	1.687	0.206	0.122	0.246
P. aeruginosa	PS24	-0.247	0.911	0.009	-0.052	0.919
P. aeruginosa	PS25	-0.207	0.321	0.09	0.077	1.114
P. aeruginosa	PS26	-0.253	0.393	0.625	0.38	0.295
P. aeruginosa	PS27	-0.134	0.461	0.26	0.132	0.291
P. aeruginosa	PS28	-0.19	0.632	0.25	0.205	0.099
P. aeruginosa	PS29	0.096	1.354	0.58	0.004	0.195
P. aeruginosa	PS30	-0.154	0.262	0.018	-0.056	1.786
P. aeruginosa	PS31	-0.053	1.253	0.288	0.1	0.251
P. aeruginosa	PS32	-0.327	0.365	0.137	-0.003	0.109

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**
P. aeruginosa	PS33	-0.31	0.343	-0.003	-0.007	1.686
P. aeruginosa	PS34	-0.057	1.417	0.178	0.039	0.252
P. aeruginosa	PS35	-0.25	0.114	0.03	-0.044	0.508
P. aeruginosa	PS36	-0.121	0.858	0.169	0.074	0.139
P. aeruginosa	PS37	-0.218	0.594	-0.003	-0.018	1.605
P. aeruginosa	PS38	-0.109	0.825	0.247	0.209	0.169
P. aeruginosa	PS39	-0.169	0.927	0.325	0.389	0.146
P. aeruginosa	PS40	-0.161	0.286	0.222	0.078	0.131
P. aeruginosa	PS41	-0.198	0.809	-0.002	-0.05	0.213
P. aeruginosa	PS42	-0.101	0.672	0.198	0.022	0.212
P. aeruginosa	PS43	-0.211	0.289	0.411	0.271	0.143
P. aeruginosa	PS44	-0.104	0.794	0.023	-0.054	0.132
P. aeruginosa	PS45	-0.134	0.669	0.05	0.037	1.16
P. aeruginosa	PS46	-0.117	0.553	0.008	-0.043	0.319
P. aeruginosa	PS47	-0.18	0.538	0.035	-0.045	0.683
P. aeruginosa	PS48	-0.145	0.066	-0.006	-0.058	1.84
P. aeruginosa	PS49	-0.201	1.584	0.332	0.352	0.229
P. aeruginosa	PS50	-0.187	0.55	0.016	-0.056	1.281
P. aeruginosa	PS51	-0.171	0.332	0.45	0.192	0.497
P. aeruginosa	PS52	-0.292	0.143	-0.02	-0.054	1.738
B. cepacia	LMG 1222	0.408	0.152	0.023	0.232	0.294
B. cepacia	LMG 2161	-0.074	0.058	0	0.051	0.04
B. cenocepacia	LMG 16654	0.148	0.127	0.018	0.103	0.215
B. cenocepacia	LMG 16656	-0.096	0.074	0.032	0.145	0.219
B. cenocepacia	LMG 16659	-0.207	0.166	0.013	0.094	0.505
B. cepacia	LMG 17997	-0.156	0.116	0.169	0.338	0.356
B. cepacia	LMG 18821	-0.007	0.042	0.023	-0.052	-0.004
B. cenocepacia	LMG 18826	-0.217	0.49	-0.114	-0.008	0.199
B. cenocepacia	LMG 18827	-0.273	0.265	-0.005	-0.054	0.183
B. cenocepacia	LMG 18828	-0.133	0.589	0.124	0.217	0.363
B. cenocepacia	LMG 18829	-0.131	0.388	0.088	0.497	0.912
B. cenocepacia	LMG 18830	-0.174	0.202	-0.029	0.07	0.113
B. cenocepacia	LMG 18832	-0.189	1.008	0.054	0.044	0.208
B. cenocepacia	LMG 18863	-0.265	0.111	0.013	-0.028	0.072
B. multivorans	LMG 13010	-0.302	0.619	0.648	0.447	0.386
B. multivorans	LMG 16660	-0.113	1.12	0.61	0.407	0.45
B. multivorans	LMG 16665	-0.127	0.322	0.051	0.274	0.369
B. multivorans	LMG 17588	-0.086	0.151	-0.011	-0.061	2.196
B. multivorans	LMG 18822	-0.023	0.139	-0.009	-0.061	2.404
B. multivorans	LMG 18823	-0.228	0.708	0.056	0.031	2.17
B. multivorans	LMG 18824	-0.116	0.11	-0.009	-0.06	0.107
B. multivorans	LMG 18825	-0.161	0.264	-0.017	-0.069	0.166
B. stabilis	LMG 14086	-0.283	0.421	0.001	0.048	0.039
B. stabilis	LMG 14294	-0.046	0.124	-0.012	-0.02	2.101
B. stabilis	LMG 18870	0.275	0.127	0.036	0.121	0.23
B. stabilis	LMG 18888	0.206	0.087	0.043	-0.02	0.073
B. vietnamiensis	LMG 10929	-0.143	0.107	0.003	-0.064	0.049
B. vietnamiensis	LMG 16232	-0.182	0.027	-0.012	-0.055	0.029
B. vietnamiensis	LMG 18835	-0.204	0.022	-0.014	-0.059	0.018
B. vietnamiensis	LMG 18836	-0.543	0.028	-0.033	-0.065	0.075
A. baumannii	ATCC 19606	-0.111	0.099	-0.007	-0.047	0.305
A. calcoaceticus	7844	-0.424	0.102	-0.005	-0.042	0.54

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**
A. haemolyticus	12155	-0.167	0.247	0.092	-0.056	0.315
A. johnsonii	10308	-0.364	0.137	-0.044	-0.059	0.808
A. lwoffi	5866	-0.43	0.077	0.006	-0.058	1.19
A. lwoffi	5867	0.061	0.391	0.085	-0.028	0.538
A. lwoffi	NCIMB 12456	0.18	0.09	0.021	-0.05	0.105
B diminuta	ATCC 11568	0.2	1.817	2.228	2.142	1.553
B. vesicularis	ATCC 11426	-0.2	2.201	2.507	9.844	2.802
R. pickettii	11149	0.015	0.142	0.518	-0.043	0.113
C. meningosepticum	ATCC 13253	0.473	2.348	1.826	0.571	9.888
M. nonliquefaciens	10464	-0.253	0.393	0.066	-0.048	2.617
M. osloensis	10465	-0.079	0.879	0.297	0.16	2.187
M. urethralis	11010	0.236	0.012	0.002	-0.06	0.006
O. urethralis	11999	-0.104	1.192	0.734	0.204	2.786
P. acidovorans	10683	0.035	-0.003	-0.006	0.009	0.256
P. aeruginosa	6749	-0.362	0.097	0.061	-0.052	0.062
P. aeruginosa	10332	-0.111	0.207	0.856	0.155	1.011
P. alcaligenes	10367	-0.127	0.264	9.896	-0.056	0.103
P. pseudoalcaligenes	10860	-0.051	0.126	0.004	-0.062	0.74
P. diminuta	8545	-0.18	2.458	9.814	2.747	2.739
P. fluorescens	10754	-0.249	2.041	8.853	0.589	2.79
P. fluorescens	10392	-0.109	1.56	2.483	-0.028	0.164
P. fluorescens	3756	-0.075	1.021	0.175	-0.048	0.6
P. fluorescens	10038	-0.061	0.711	0.195	-0.043	0.286
P. fluorescens	10688	-0.099	1.09	0.062	-0.005	2.775
P. fluorescens	9428	0.125	0.249	2.395	0.184	2.078
P. fragi	NCIMB 8987	0.126	0.072	0.014	-0.06	0.132
P. maltophilia	10257	-0.289	1.564	2.236	0.003	0.075
P. paucimobilis	11030	-0.171	1.185	1.722	0.352	0.872
R. pickettii	11149	-0.133	0.134	0.588	-0.043	0.28
P. putida	10936	-0.196	0.579	9.912	0.015	2.759
P. stutzeri	12262	-0.23	0.615	0.403	-0.01	1.454
P. stutzeri	10475	-0.128	0.378	2.566	-0.019	0.448
P. vesiculare	10900	-0.156	2.172	9.885	2.135	2.665
S. spiritivorum	ATCC 33861	0.238	0.925	0.167	0.017	1.185
B. ambifaria	11351	0.225	0.372	0.245	1.015	0.274
B. andropogonis	1279	-0.238	1.858	2.479	0.008	0.091
B. andropogonis	2126	0.204	1.333	1.489	0.265	2.699
B. caryphylli	2155	-0.047	0.295	0.461	0.024	1.034
B. caryphylli	2156	0.012	0.278	0.021	-0.044	0.594
B. dolosa	18941	0.053	0.068	0.049	-0.046	0.446
B. dolosa	18942	0.041	0.907	0.385	0.036	2.567
B. gladioli	11626	-0.089	1.183	0.223	-0.049	0.723
B. gladioli	18113	-0.024	0.018	-0.004	-0.062	0.192
B.gladioli pv. alliicola	2121	-0.069	0.282	0.08	-0.064	0.491
B. gladioli pv. alliicola	6877	-0.022	0.099	0.006	-0.058	0.434
B.gladioli pv. gladioli	2216	-0.016	0.062	0.018	-0.052	0.444
B.gladioli pv. gladioli	6880	0.096	0.299	0.054	-0.051	0.148
B. glumae	1277	0.183	0.158	0.048	-0.059	0.7
B. glumae	2196	0.139	0.106	0.145	-0.062	0.602
B. phenazinium	2247	-0.129	0.727	0.27	0.025	2.692
B. phenazinium	6868	-0.201	1.258	1.073	0.107	2.499
P. apista	16408	-0.126	0.037	-0.002	-0.053	0.023

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**
P. norimberensis	13019	0.26	1.551	1.271	0.28	9.885
P. norimberensis	16603	0.323	0.001	0.004	-0.055	0.004
P. pnomenusa	18087	0.19	0.294	0.008	-0.051	0.004
P. pnomenusa	18817	0.138	0.195	0.558	-0.053	0.034
P. pulmonicola	18107	0.321	0.086	0.037	-0.059	0.046
P. sputorum	18100	0.172	0.011	-0.005	-0.057	0.013
P. sputorum	18819	0.139	0.076	0.034	-0.059	0.024
R. basilensis	18990	0.077	0.116	0.048	-0.058	0.09
R. basilensis	19286	0.1	0.217	0.148	-0.052	0.296
R. campinensis	19282	-0.091	0.2	-0.028	-0.06	0.143
R. campinensis	19283	0.361	0.015	0.01	-0.044	0.153
R. eutropha	1190	0.035	0.941	2.632	0.09	1.724
R. eutropha	1194	0.411	0.037	0.004	-0.061	0.08
R. gilardii	3399	0.033	0.022	0.031	-0.045	0.121
R. gilardii	3400	0.156	0.225	0.044	-0.045	0.085
R. mannitolilytica	19090	0.336	1.548	1.337	0.274	2.83
R. metallidurans	1195	0.125	0.488	0.235	-0.03	0.298
R. metallidurans	19290	0.101	0.213	0.087	-0.076	0.179
R. paucula	3244	0.082	0.821	0.156	-0.018	1.137
R. paucula	3245	0.239	0.078	0.055	-0.07	0.181
R. pickettii	5942	0.219	0.16	0.095	-0.064	0.178
R. pickettii	6871	0.133	0.532	0.459	-0.035	0.284
R. solanacearum	2291	0.028	0.362	0.068	-0.036	0.735
R. solanacearum	2293	0.193	1.523	0.409	0.109	2.174
R. taiwanensis	19425	-0.049	1.686	1.26	0.359	2.698
S. maltophilia	957	-0.3	1.562	9.892	0.082	0.076
S. maltophilia	958	-0.269	2.17	2.304	0.051	0.088
H_2O		0.236	0.004	0.001	-0.058	0.002
H_2O		0.326	-0.003	0.003	-0.059	0.004
H_2O		0.429	0.046	0.004	-0.055	0.004
H_2O		0.23	0.003	-0.001	-0.055	0.004
H_2O		0.619	0.005	0.001	-0.067	0.001
E. coli	10418	-0.126	0.102	-0.011	-0.038	0.157
Enterobacter cloacae	11936	-0.239	0.131	-0.015	-0.021	0.161

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**F**	**G**	**H**	**I**	**J**
P. aeruginosa	2688	0.496	-0.028	0.138	0.141	0.643
P. aeruginosa	2702	0.185	-0.014	0.093	0.067	1.513
P. aeruginosa	2704	0.052	-0.015	0.22	0.101	1.317
P. aeruginosa	2706	0.159	-0.005	0.118	0.014	0.89
P. aeruginosa	2715	0.139	0.044	0.104	-0.007	2.344
P. aeruginosa	2720	0.244	-0.044	0.154	0.318	0.513
P. aeruginosa	2737	0.107	0.005	0.236	0.076	1.823
P. aeruginosa	2739	0.134	0.026	0.158	0.079	1.995
P. aeruginosa	2741	0.138	-0.036	0.153	0.165	0.215
P. aeruginosa	2742	0.145	0.008	0.339	0.056	0.775
P. aeruginosa	2749	0.185	0.001	0.331	0.079	0.867
P. aeruginosa	2772	0.452	0.061	0.098	0.052	1.418
P. aeruginosa	2775	0.126	-0.063	0.35	0.081	0.921
P. aeruginosa	2776	0.285	0.015	0.318	0.769	1.515
P. aeruginosa	2778	0.213	-0.04	0.205	0.461	1.254
P. aeruginosa	2779	0.067	0.172	0.11	0.057	0.254
P. aeruginosa	2780	0.026	0.004	0.107	0.173	0.767
P. aeruginosa	2781	0.013	-0.053	0.386	0.833	0.477
P. aeruginosa	2782	0.228	0.021	0.535	0.254	1.012
P. aeruginosa	2783	0.141	-0.025	0.498	0.687	1.318
P. aeruginosa	PS1	-0.024	-0.045	0.228	0.033	0.303
P. aeruginosa	PS2	0.09	-0.028	0.191	0.516	0.519
P. aeruginosa	PS3	0.137	-0.003	0.222	0.032	1.671
P. aeruginosa	PS4	0.007	-0.02	0.116	0.044	1.036
P. aeruginosa	PS5	0.339	0	0.146	0.106	1.836
P. aeruginosa	PS6	0.48	-0.005	0.117	0.277	1.574
P. aeruginosa	PS7	0.249	-0.014	0.294	-0.003	0.372
P. aeruginosa	PS8	0.151	-0.049	0.087	0.11	0.431
P. aeruginosa	PS9	0.169	-0.041	0.251	1.028	2.074
P. aeruginosa	PS10	-0.028	-0.019	0.173	0.288	0.962
P. aeruginosa	PS11	0.252	-0.047	0.226	0.08	0.305
P. aeruginosa	PS12	0.079	-0.018	0.208	0.103	0.764
P. aeruginosa	PS13	0.075	0.023	0.244	0.108	2.158
P. aeruginosa	PS14	0.272	0.054	0.188	0.059	1.959
P. aeruginosa	PS15	0.24	0.003	0.119	0.013	2.383
P. aeruginosa	PS16	0.087	0.081	0.118	0.078	0.937
P. aeruginosa	PS17	0.157	-0.017	0.119	0.222	0.719
P. aeruginosa	PS18	0.083	-0.01	0.204	1.232	1.13
P. aeruginosa	PS19	0.243	0.012	0.134	0.154	1.121
P. aeruginosa	PS20	0.204	0.007	0.144	0.039	1.794
P. aeruginosa	PS21	0.163	0.019	0.112	0.005	0.994
P. aeruginosa	PS22	0.247	-0.05	0.128	0.127	1.543
P. aeruginosa	PS23	0.104	-0.01	0.182	0.763	1.828
P. aeruginosa	PS24	0.189	0.039	0.163	0.102	0.788
P. aeruginosa	PS25	0.327	-0.018	0.117	0.002	0.325
P. aeruginosa	PS26	0.048	-0.064	0.217	0.215	1.459
P. aeruginosa	PS27	0.149	0.018	0.205	0.182	0.638
P. aeruginosa	PS28	0.116	-0.064	0.26	0.112	1.244
P. aeruginosa	PS29	0.023	-0.016	0.19	0.005	1.045
P. aeruginosa	PS30	0.329	-0.024	0.148	-0.033	0.798
P. aeruginosa	PS31	0.051	-0.017	0.157	-0.013	0.787
P. aeruginosa	PS32	0.071	0.083	0.127	-0.022	1.066

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**F**	**G**	**H**	**I**	**J**
P. aeruginosa	PS33	-0.067	-0.025	0.207	0.04	0.169
P. aeruginosa	PS34	0.156	-0.017	0.144	0.122	1.07
P. aeruginosa	PS35	0.267	-0.091	0.113	0.051	0.211
P. aeruginosa	PS36	0.089	-0.027	0.119	0.001	1.215
P. aeruginosa	PS37	0.184	-0.017	0.146	-0.023	1.399
P. aeruginosa	PS38	0.042	-0.022	0.141	0.029	1.286
P. aeruginosa	PS39	0.151	0.001	0.14	0.059	1.383
P. aeruginosa	PS40	0.744	0.01	0.118	0.199	0.842
P. aeruginosa	PS41	0.09	-0.029	0.137	0.433	0.655
P. aeruginosa	PS42	0.438	-0.04	0.122	0.046	1.24
P. aeruginosa	PS43	0.371	0.019	0.139	0.051	1.128
P. aeruginosa	PS44	0.177	-0.004	0.138	0.053	1.356
P. aeruginosa	PS45	0.206	0.013	0.166	-0.62	1.783
P. aeruginosa	PS46	-0.012	-0.026	0.225	0.042	1.001
P. aeruginosa	PS47	0.185	-0.023	0.158	0.084	1.414
P. aeruginosa	PS48	0.424	-0.007	0.125	0.039	0.504
P. aeruginosa	PS49	0.153	-0.055	0.301	0.08	2.041
P. aeruginosa	PS50	0.103	-0.013	0.187	0.074	1.163
P. aeruginosa	PS51	0.134	-0.009	0.27	0.134	0.59
P. aeruginosa	PS52	0.109	-0.076	0.114	0.087	0.385
B. cepacia	LMG 1222	0.88	0.008	0.268	-0.006	0.141
B. cepacia	LMG 2161	0.392	-0.021	0.1	0.28	0.066
B. cenocepacia	LMG 16654	0.603	-0.035	0.228	-0.023	0.115
B. cenocepacia	LMG 16656	0.353	-0.063	0.221	-0.016	0.111
B. cenocepacia	LMG 16659	0.199	-0.004	0.114	0.006	0.108
B. cepacia	LMG 17997	0.482	-0.066	0.563	0.254	0.475
B. cepacia	LMG 18821	0.619	-0.024	0.087	0.073	0.674
B. cenocepacia	LMG 18826	0.458	-0.044	0.132	0.217	0.419
B. cenocepacia	LMG 18827	-0.006	-0.083	0.116	0.007	1.015
B. cenocepacia	LMG 18828	-0.008	-0.039	0.296	0.066	0.836
B. cenocepacia	LMG 18829	0.249	-0.021	0.618	0.01	0.049
B. cenocepacia	LMG 18830	0.393	-0.107	0.136	0.142	0.788
B. cenocepacia	LMG 18832	0.417	-0.033	0.238	0.029	0.266
B. cenocepacia	LMG 18863	0.262	-0.076	0.178	0.023	0.026
B. multivorans	LMG 13010	0.573	-0.039	0.212	0.19	0.763
B. multivorans	LMG 16660	0.36	0.016	0.222	1.814	0.867
B. multivorans	LMG 16665	0.211	-0.076	0.403	0.439	0.418
B. multivorans	LMG 17588	1.096	-0.083	1.283	-0.028	0.157
B. multivorans	LMG 18822	0.65	-0.09	2.191	-0.025	0.127
B. multivorans	LMG 18823	0.849	-0.034	0.815	-0.021	0.725
B. multivorans	LMG 18824	0.29	-0.043	0.159	-0.03	0.037
B. multivorans	LMG 18825	0.273	-0.027	0.301	-0.061	0.221
B. stabilis	LMG 14086	0.32	-0.024	0.091	-0.005	0.106
B. stabilis	LMG 14294	0.501	-0.135	1.216	0.018	0.558
B. stabilis	LMG 18870	0.762	0.065	0.226	0.037	0.09
B. stabilis	LMG 18888	0.295	-0.053	0.145	2.82	0.024
B. vietnamiensis	LMG 10929	0.535	0.07	0.206	2.038	0.231
B. vietnamiensis	LMG 16232	0.453	-0.071	0.095	0.859	0.014
B. vietnamiensis	LMG 18835	0.409	-0.05	0.159	0.023	0.002
B. vietnamiensis	LMG 18836	0.83	-0.126	0.476	-0.043	0.157
A. baumannii	ATCC 19606	0.629	-0.111	0.344	0.023	0.136
A. calcoaceticus	7844	0.636	-0.05	0.394	-0.029	0.103

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**F**	**G**	**H**	**I**	**J**
A. haemolyticus	12155	0.173	0.02	0.388	-0.022	0.373
A. johnsonii	10308	1.128	0.21	0.666	0.045	0.048
A. lwoffi	5866	0.533	-0.097	0.245	-0.016	0.094
A. lwoffi	5867	0.921	0.046	0.345	0.046	0.126
A. lwoffi	NCIMB 12456	0.714	-0.088	0.127	-0.007	0.067
B diminuta	ATCC 11568	0.648	-0.1	1.659	-0.004	1.731
B. vesicularis	ATCC 11426	0.729	-0.04	2.769	0.022	1.993
R. pickettii	11149	0.683	-0.087	0.246	-0.052	0.151
C. meningosepticum	ATCC 13253	9.358	-0.1	9.863	0.073	2.297
M. nonliquefaciens	10464	0.143	-0.03	0.181	-0.004	0.22
M. osloensis	10465	0.926	-0.033	1.571	0.107	2.203
M. urethralis	11010	0.211	-0.067	0.13	-0.03	0.029
O. urethralis	11999	1.656	-0.005	2.788	0.088	2.052
P. acidovorans	10683	0.498	-0.096	0.155	2.634	-0.01
P. aeruginosa	6749	0.105	-0.065	0.192	9.901	0.249
P. aeruginosa	10332	0.487	0.002	0.483	0.144	0.121
P. alcaligenes	10367	0.087	-0.059	0.022	9.871	0.511
P. pseudoalcaligenes	10860	0.178	-0.067	0.751	0.46	0.246
P. diminuta	8545	0.328	-0.053	2.67	9.874	2.83
P. fluorescens	10754	1.756	0.035	2.789	1.523	1.406
P. fluorescens	10392	0.194	0.017	0.293	0.02	1.268
P. fluorescens	3756	0.414	-0.025	1.067	9.889	0.919
P. fluorescens	10038	0.272	-0.059	0.213	0.869	0.415
P. fluorescens	10688	0.129	0.001	2.707	0.002	1.289
P. fluorescens	9428	0.182	-0.014	2.225	0.601	0.173
P. fragi	NCIMB 8987	0.517	-0.014	0.084	0.548	0.059
P. maltophilia	10257	0.476	-0.015	1.742	0.29	1.817
P. paucimobilis	11030	0.429	0.046	0.867	2.795	1.711
R. pickettii	11149	0.46	-0.122	0.254	1.125	0.105
P. putida	10936	0.184	0.005	2.84	0.036	0.117
P. stutzeri	12262	0.214	0.021	1.09	0.339	0.405
P. stutzeri	10475	0.353	-0.006	0.599	9.859	2.157
P. vesiculare	10900	0.201	-0.035	9.894	1.473	2.431
S. spiritivorum	ATCC 33861	0.644	-0.039	0.284	0.025	0.778
B. ambifaria	11351	0.358	-0.05	0.33	0.061	0.212
B. andropogonis	1279	0.354	-0.03	2.074	9.906	1.472
B. andropogonis	2126	0.933	-0.055	2.694	0.236	1.013
B. caryphylli	2155	0.568	-0.025	0.889	9.847	0.729
B. caryphylli	2156	0.211	-0.125	0.649	0.464	0.026
B. dolosa	18941	0.373	-0.198	0.17	0.054	0.024
B. dolosa	18942	1.031	-0.093	2.556	1.438	0.016
B. gladioli	11626	0.45	-0.06	2.742	1.485	0.018
B. gladioli	18113	0.192	-0.107	1.396	-0.04	0.085
B.gladioli pv. alliicola	2121	0.296	-0.099	2.383	0.27	0.204
B. gladioli pv. alliicola	6877	0.169	-0.065	2.191	0.058	0.041
B.gladioli pv. gladioli	2216	0.436	-0.087	2.401	9.895	0.115
B.gladioli pv. gladioli	6880	0.167	-0.063	1.728	0.059	0.034
B. glumae	1277	0.422	-0.055	1.277	2.676	0.125
B. glumae	2196	0.267	-0.018	2.393	0.285	0.156
B. phenazinium	2247	1.038	0.035	2.604	0.683	1.065
B. phenazinium	6868	1.138	0.317	2.433	-0.032	1.707
P. apista	16408	0.353	-0.114	0.127	2.112	0.085

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**F**	**G**	**H**	**I**	**J**
P. norimberensis	13019	2.408	-0.183	2.653	0.038	0.267
P. norimberensis	16603	0.446	-0.074	0.179	0.024	0.048
P. pnomenusa	18087	0.326	-0.096	0.117	0.12	0.003
P. pnomenusa	18817	0.311	-0.126	0.231	-0.029	0.007
P. pulmonicola	18107	0.224	-0.028	0.125	0.176	0.142
P. sputorum	18100	0.515	-0.005	0.197	-0.027	0.016
P. sputorum	18819	0.279	-0.077	0.119	9.869	0.237
R. basilensis	18990	0.234	-0.055	0.106	0.065	0.07
R. basilensis	19286	0.232	-0.026	0.311	0.333	0.13
R. campinensis	19282	0.679	-0.131	0.229	-0.062	0.126
R. campinensis	19283	0.125	-0.046	0.089	0.018	0.08
R. eutropha	1190	0.974	-0.07	1.259	-0.018	1.132
R. eutropha	1194	0.362	-0.017	0.081	0.115	0.004
R. gilardii	3399	0.256	-0.062	0.194	0.105	-0.023
R. gilardii	3400	0.494	-0.064	0.104	0.179	0.01
R. mannitolilytica	19090	9.42	-0.082	2.831	0.206	0.99
R. metallidurans	1195	0.549	-0.046	0.147	0.093	0.094
R. metallidurans	19290	0.375	-0.077	0.119	0.036	0.145
R. paucula	3244	0.545	-0.058	0.932	0.727	0.005
R. paucula	3245	0.399	0.035	0.269	0.051	0.295
R. pickettii	5942	0.495	-0.082	0.119	-0.022	0.069
R. pickettii	6871	0.661	-0.104	0.179	0.002	0.072
R. solanacearum	2291	-0.082	-0.056	0.558	0.143	0.068
R. solanacearum	2293	1.31	0.313	1.592	0.043	0.025
R. taiwanensis	19425	9.407	0.136	2.663	2.017	0.767
S. maltophilia	957	0.216	-0.053	1.529	0.866	2.459
S. maltophilia	958	0.243	-0.06	1.822	0.096	1.886
H_2O		0.664	-0.039	0.137	0.032	0.01
H_2O		0.42	-0.012	0.126	0.022	0.01
H_2O		0.322	-0.036	0.121	0.045	0.016
H_2O		0.505	0.069	0.122	0.028	0.012
H_2O		0.397	0.242	0.122	0.079	0.008
E. coli	10418	0.588	0.047	0.203	0.055	0.069
Enterobacter cloacae	11936	0.179	-0.071	0.201	-0.008	0.164

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**K**	**L**	**M**	**N**	**O**
P. aeruginosa	2688	943	110	0.026	0.652	0.874
P. aeruginosa	2702	1303	-46	0.13	0.652	0.779
P. aeruginosa	2704	1187	571	0.08	0.652	0.726
P. aeruginosa	2706	903	549	0.039	0.652	0.699
P. aeruginosa	2715	2576	651	0.404	0.652	0.866
P. aeruginosa	2720	1093	528	0.012	0.652	0.631
P. aeruginosa	2737	1629	537	0.127	0.652	0.675
P. aeruginosa	2739	1956	531	0.107	0.652	1.022
P. aeruginosa	2741	138	332	0.03	0.652	0.843
P. aeruginosa	2742	671	754	0.024	0.652	0.84
P. aeruginosa	2749	1599	446	0.11	0.652	0.886
P. aeruginosa	2772	1984	628	0.065	0.652	1.108
P. aeruginosa	2775	1343	629	0.009	0.652	0.624
P. aeruginosa	2776	1966	406	0.074	0.652	1.042
P. aeruginosa	2778	1322	442	0.082	0.652	0.727
P. aeruginosa	2779	595	299	0.008	0.652	1.018
P. aeruginosa	2780	1465	385	0.025	0.652	1.068
P. aeruginosa	2781	769	272	0.1	0.652	0.71
P. aeruginosa	2782	1703	403	0.038	0.652	0.934
P. aeruginosa	2783	2231	488	0.055	0.652	0.806
P. aeruginosa	PS1	1267	244	0.014	0.652	0.701
P. aeruginosa	PS2	778	336	0.015	0.652	0.844
P. aeruginosa	PS3	1675	467	0.068	0.652	1.303
P. aeruginosa	PS4	784	391	0.047	0.652	1.13
P. aeruginosa	PS5	1733	509	0.18	0.652	0.628
P. aeruginosa	PS6	1206	547	0.438	0.652	1.26
P. aeruginosa	PS7	390	339	0.231	0.652	0.564
P. aeruginosa	PS8	195	257	0.024	0.652	0.538
P. aeruginosa	PS9	1780	495	0.106	0.652	1.378
P. aeruginosa	PS10	1083	339	0.342	0.652	1.002
P. aeruginosa	PS11	524	216	0.052	0.652	0.702
P. aeruginosa	PS12	1065	248	0.034	0.652	0.723
P. aeruginosa	PS13	1337	525	0.09	0.652	0.613
P. aeruginosa	PS14	1944	583	0.038	0.652	0.561
P. aeruginosa	PS15	1572	601	0.131	0.652	0.628
P. aeruginosa	PS16	1306	525	0.051	0.652	0.5
P. aeruginosa	PS17	1654	405	1.107	0.652	0.829
P. aeruginosa	PS18	973	537	0.644	0.652	0.761
P. aeruginosa	PS19	772	482	0.827	0.652	0.963
P. aeruginosa	PS20	6666	577	1.398	0.652	0.789
P. aeruginosa	PS21	818	489	0.952	0.652	0.774
P. aeruginosa	PS22	1545	342	0.494	0.652	0.781
P. aeruginosa	PS23	1578	607	0.085	0.652	0.8
P. aeruginosa	PS24	3503	598	0.054	0.652	1.174
P. aeruginosa	PS25	1117	330	0.047	0.652	1.011
P. aeruginosa	PS26	3410	382	0.486	0.652	2.333
P. aeruginosa	PS27	1962	516	0.043	0.652	0.963
P. aeruginosa	PS28	1899	576	0.086	0.652	0.728
P. aeruginosa	PS29	568	558	0.636	0.652	0.693
P. aeruginosa	PS30	2121	562	0.866	0.652	0.723
P. aeruginosa	PS31	543	369	0.506	0.652	0.763
P. aeruginosa	PS32	1330	431	0.895	0.652	0.732

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**K**	**L**	**M**	**N**	**O**
P. aeruginosa	PS33	1877	210	0.328	0.652	0.746
P. aeruginosa	PS34	785	366	0.759	0.652	0.716
P. aeruginosa	PS35	128	247	0.577	0.652	0.698
P. aeruginosa	PS36	1254	315	0.565	0.652	0.868
P. aeruginosa	PS37	2438	507	0.919	0.652	0.71
P. aeruginosa	PS38	1124	504	0.531	0.652	1.473
P. aeruginosa	PS39	1498	442	0.742	0.652	1.133
P. aeruginosa	PS40	686	271	0.057	0.652	1.157
P. aeruginosa	PS41	546	229	0.918	0.652	0.77
P. aeruginosa	PS42	964	534	0.817	0.652	0.82
P. aeruginosa	PS43	1617	620	1.098	0.652	0.852
P. aeruginosa	PS44	2298	730	9.459	0.652	0.719
P. aeruginosa	PS45	1843	608	1.11	0.652	1.543
P. aeruginosa	PS46	498	231	1.124	0.652	1.105
P. aeruginosa	PS47	1233	622	1.308	0.652	0.87
P. aeruginosa	PS48	2151	445	0.626	0.652	0.838
P. aeruginosa	PS49	1630	320	0.762	0.652	0.792
P. aeruginosa	PS50	689	555	1.343	0.652	0.899
P. aeruginosa	PS51	1145	333	0.436	0.652	0.872
P. aeruginosa	PS52	2759	379	0.754	0.652	0.718
B. cepacia	LMG 1222	858	763	0.301	0.652	1.174
B. cepacia	LMG 2161	52	553	0.561	0.652	1.379
B. cenocepacia	LMG 16654	357	714	0.303	0.652	1.114
B. cenocepacia	LMG 16656	467	736	0.278	0.652	1.09
B. cenocepacia	LMG 16659	210	580	0.466	0.652	0.723
B. cepacia	LMG 17997	128	775	0.293	0.652	0.872
B. cepacia	LMG 18821	1044	684	0.588	0.652	0.829
B. cenocepacia	LMG 18826	2191	689	0.482	0.652	1.434
B. cenocepacia	LMG 18827	4764	418	1.26	0.652	1.57
B. cenocepacia	LMG 18828	736	724	0.982	0.652	0.88
B. cenocepacia	LMG 18829	-12	620	0.252	0.652	0.855
B. cenocepacia	LMG 18830	1031	659	1.201	0.652	0.727
B. cenocepacia	LMG 18832	146	467	0.153	0.652	0.9
B. cenocepacia	LMG 18863	-131	803	0.224	0.652	0.75
B. multivorans	LMG 13010	1117	632	0.446	0.652	1.055
B. multivorans	LMG 16660	821	509	0.841	0.652	0.773
B. multivorans	LMG 16665	903	501	1.007	0.652	0.871
B. multivorans	LMG 17588	1093	718	0.271	0.652	0.69
B. multivorans	LMG 18822	1407	726	0.35	0.652	0.831
B. multivorans	LMG 18823	1127	644	0.549	0.652	0.779
B. multivorans	LMG 18824	311	351	0.278	0.652	0.796
B. multivorans	LMG 18825	4087	711	1.358	0.652	0.868
B. stabilis	LMG 14086	2106	678	0.225	0.652	0.77
B. stabilis	LMG 14294	1950	357	1.548	0.652	0.802
B. stabilis	LMG 18870	690	400	0.052	0.652	1.042
B. stabilis	LMG 18888	348	257	0.013	0.652	1.195
B. vietnamiensis	LMG 10929	28003	391	0.23	0.652	0.756
B. vietnamiensis	LMG 16232	687	86	-0.014	0.652	0.879
B. vietnamiensis	LMG 18835	708	101	-0.022	0.652	0.908
B. vietnamiensis	LMG 18836	1722	431	0.125	0.652	0.964
A. baumannii	ATCC 19606	4121	519	0.032	0.652	0.962
A. calcoaceticus	7844	3928	479	0.057	0.652	0.789

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**K**	**L**	**M**	**N**	**O**
A. haemolyticus	12155	1776	507	0.048	0.652	0.664
A. johnsonii	10308	2435	451	0.117	0.652	0.813
A. lwoffi	5866	1547	443	-0.019	0.652	0.98
A. lwoffi	5867	9742	644	0.035	0.652	1.069
A. lwoffi	NCIMB 12456	-40	40	0.011	0.652	0.816
B diminuta	ATCC 11568	39426	464	1.747	0.652	0.751
B. vesicularis	ATCC 11426	39362	421	1.636	0.652	0.682
R. pickettii	11149	1462	345	0.24	0.652	1.639
C. meningosepticum	ATCC 13253	36712	836	1.039	0.652	0.91
M. nonliquefaciens	10464	4810	473	0.24	0.652	0.902
M. osloensis	10465	30722	760	1.064	0.652	1.087
M. urethralis	11010	625	394	-0.003	0.652	0.711
O. urethralis	11999	27508	684	0.83	0.652	1.178
P. acidovorans	10683	821	373	-0.007	0.652	0.726
P. aeruginosa	6749	4728	559	0.181	0.652	0.938
P. aeruginosa	10332	3796	446	-0.002	0.652	0.537
P. alcaligenes	10367	1037	91	0.931	0.652	1.298
P. pseudoalcaligenes	10860	244	195	0.037	0.652	0.535
P. diminuta	8545	38916	186	1.282	0.652	0.566
P. fluorescens	10754	18126	525	0.703	0.652	0.819
P. fluorescens	10392	5866	522	0.624	0.652	0.588
P. fluorescens	3756	5841	467	0.344	0.652	0.669
P. fluorescens	10038	1804	381	0.098	0.652	0.45
P. fluorescens	10688	5466	418	0.708	0.652	0.592
P. fluorescens	9428	4392	360	0.377	0.652	1.103
P. fragi	NCIMB 8987	970	369	-0.019	0.652	0.128
P. maltophilia	10257	25466	94	1.222	0.652	0.682
P. paucimobilis	11030	38141	799	2.568	0.652	0.909
R. pickettii	11149	1153	314	0.182	0.652	2.1
P. putida	10936	1392	376	0.068	0.652	0.663
P. stutzeri	12262	2073	412	0.033	0.652	0.754
P. stutzeri	10475	6135	467	1.321	0.652	0.781
P. vesiculare	10900	39081	385	2.316	0.652	0.775
S. spiritivorum	ATCC 33861	39051	494	0.34	0.652	1.002
B. ambifaria	11351	3217	332	0.033	0.652	0.985
B. andropogonis	1279	26879	79	1.533	0.652	0.727
B. andropogonis	2126	33685	519	0.854	0.652	1.297
B. caryphylli	2155	15299	601	0.718	0.652	0.924
B. caryphylli	2156	18	58	0.01	0.652	-0.075
B. dolosa	18941	-61	58	0.04	0.652	-0.077
B. dolosa	18942	-95	52	0.01	0.652	-0.084
B. gladioli	11626	-103	67	0.005	0.652	-0.086
B. gladioli	18113	449	302	0.028	0.652	0.663
B.gladioli pv. alliicola	2121	784	446	0.333	0.652	0.863
B. gladioli pv. alliicola	6877	302	321	0.064	0.652	0.81
B.gladioli pv. gladioli	2216	1230	376	0.062	0.652	0.92
B.gladioli pv. gladioli	6880	73	83	0.002	0.652	1.049
B. glumae	1277	485	467	0.015	0.652	1.114
B. glumae	2196	305	351	0.045	0.652	1.429
B. phenazinium	2247	10813	562	1.161	0.652	1.134
B. phenazinium	6868	31899	647	0.932	0.652	0.668
P. apista	16408	101	95	-0.001	0.652	0.899

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**K**	**L**	**M**	**N**	**O**
P. norimberensis	13019	2234	275	0.129	0.652	0.746
P. norimberensis	16603	-46	141	0.16	0.652	0.796
P. pnomenusa	18087	18	104	0.026	0.652	0.195
P. pnomenusa	18817	3	152	-0.009	0.652	0.311
P. pulmonicola	18107	-79	67	0.041	0.652	0.627
P. sputorum	18100	-9	198	-0.013	0.652	0.575
P. sputorum	18819	3153	498	0.214	0.652	0.894
R. basilensis	18990	269	506	0.026	0.652	0.672
R. basilensis	19286	647	189	0.078	0.652	0.564
R. campinensis	19282	5045	445	-0.045	0.652	0.863
R. campinensis	19283	7923	433	0.148	0.652	0.605
R. eutropha	1190	38431	543	0.962	0.652	1.236
R. eutropha	1194	586	262	-0.007	0.652	0.614
R. gilardii	3399	3706	467	0.007	0.652	0.566
R. gilardii	3400	1270	351	-0.035	0.652	0.573
R. mannitolilytica	19090	20580	534	0.327	0.652	0.783
R. metallidurans	1195	348	202	-0.02	0.652	0.787
R. metallidurans	19290	1737	403	0.125	0.652	0.713
R. paucula	3244	717	229	-0.022	0.652	0.955
R. paucula	3245	5441	528	0.196	0.652	0.867
R. pickettii	5942	1013	354	-0.011	0.652	2.012
R. pickettii	6871	1102	271	0.078	0.652	1.337
R. solanacearum	2291	629	406	1.157	0.652	0.661
R. solanacearum	2293	1346	327	0.655	0.652	0.732
R. taiwanensis	19425	13463	513	0.438	0.652	0.867
S. maltophilia	957	38709	400	2.823	0.652	0.677
S. maltophilia	958	38221	95	0.848	0.652	0.782
H_2O		-49	46	0.004	0.652	-0.088
H_2O		-15	22	0.012	0.652	-0.034
H_2O		-43	37	0.006	0.652	-0.086
H_2O		-82	18	0.007	0.652	-0.083
H_2O		-25	25	0.001	0.652	-0.081
E. coli	10418	842	384	-0.002	0.652	-0.019
Enterobacter cloacae	11936	2762	482	-0.009	0.652	0.055

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**
P. aeruginosa	2688	-0.001	0.576	1.411	0.209	0.013
P. aeruginosa	2702	0.013	0.889	1.566	0.081	0.01
P. aeruginosa	2704	0.002	0.594	1.478	0.033	0.003
P. aeruginosa	2706	0.001	0.518	1.62	0.075	0.001
P. aeruginosa	2715	0.02	0.573	1.664	0.12	-0.021
P. aeruginosa	2720	-0.001	0.346	1.55	0.08	-0.311
P. aeruginosa	2737	-0.003	0.491	1.691	0.151	0.012
P. aeruginosa	2739	0.001	0.387	1.534	0.024	-0.001
P. aeruginosa	2741	0.031	0.696	1.802	0.148	-0.043
P. aeruginosa	2742	0.014	0.463	1.608	-0.35	0.063
P. aeruginosa	2749	0.047	0.627	1.602	0.208	-0.045
P. aeruginosa	2772	0.011	0.563	1.947	-0.056	-0.001
P. aeruginosa	2775	0.004	0.587	1.181	0.09	-0.004
P. aeruginosa	2776	0.008	0.586	1.617	0.161	-0.009
P. aeruginosa	2778	0.029	0.416	1.435	0.21	0.017
P. aeruginosa	2779	0.011	0.688	1.696	0.067	-0.02
P. aeruginosa	2780	0.006	0.472	1.625	0.059	0.005
P. aeruginosa	2781	0.015	0.39	1.744	0.138	-0.026
P. aeruginosa	2782	0.021	0.644	1.695	0.127	-0.011
P. aeruginosa	2783	0.017	0.454	1.509	0.154	-0.005
P. aeruginosa	PS1	0.001	0.552	1.771	0.136	-0.005
P. aeruginosa	PS2	0.014	0.642	1.537	0.172	-0.027
P. aeruginosa	PS3	0.014	0.612	1.747	0.036	0.007
P. aeruginosa	PS4	0.008	0.503	1.465	0.059	0.007
P. aeruginosa	PS5	0.004	0.426	1.48	0.098	0.047
P. aeruginosa	PS6	0.008	0.694	1.525	0	-0.003
P. aeruginosa	PS7	0.016	0.57	1.621	0.111	0.021
P. aeruginosa	PS8	0.004	0.665	1.759	0.128	0.031
P. aeruginosa	PS9	0.018	0.577	1.675	0.139	0.022
P. aeruginosa	PS10	0.018	0.594	1.957	0.122	0.001
P. aeruginosa	PS11	0.025	0.591	1.683	0.219	-0.032
P. aeruginosa	PS12	0.002	0.492	1.608	0.09	0.017
P. aeruginosa	PS13	-0.007	1.022	1.778	0.005	0.011
P. aeruginosa	PS14	0.037	0.546	1.66	0.032	-0.007
P. aeruginosa	PS15	-0.002	0.543	1.928	-0.029	0.001
P. aeruginosa	PS16	0.009	0.305	1.623	0.102	-0.002
P. aeruginosa	PS17	0.035	0.529	1.424	0.123	0.004
P. aeruginosa	PS18	0.015	0.917	1.406	0.086	0.009
P. aeruginosa	PS19	0.004	0.522	1.578	0.092	0.012
P. aeruginosa	PS20	0.006	0.739	1.616	-0.022	0.01
P. aeruginosa	PS21	0.002	0.561	1.581	0.132	-0.012
P. aeruginosa	PS22	0.043	0.904	1.755	0.151	0.03
P. aeruginosa	PS23	-0.01	0.685	1.649	0.009	-0.001
P. aeruginosa	PS24	0.018	0.575	1.459	0.106	0.013
P. aeruginosa	PS25	0.032	0.635	1.838	-0.094	-0.005
P. aeruginosa	PS26	0.049	0.598	1.835	0.168	-0.003
P. aeruginosa	PS27	0.031	0.619	1.758	0.185	-0.013
P. aeruginosa	PS28	0.064	0.642	1.605	0.144	-0.017
P. aeruginosa	PS29	-0.001	0.613	1.372	0.02	0.011
P. aeruginosa	PS30	0.013	0.529	1.488	0.132	0.002
P. aeruginosa	PS31	0.001	1.029	1.485	0.113	0.012
P. aeruginosa	PS32	0.012	0.631	1.49	0.111	0.006

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**
P. aeruginosa	PS33	-0.017	0.503	1.675	0.156	-0.012
P. aeruginosa	PS34	0.005	0.233	1.877	0.187	0.027
P. aeruginosa	PS35	0.059	0.61	2.032	0.197	0.015
P. aeruginosa	PS36	0.021	0.773	1.818	0.171	0.012
P. aeruginosa	PS37	0.003	0.65	1.964	-0.054	-0.013
P. aeruginosa	PS38	0.007	0.4	1.684	0.032	-0.002
P. aeruginosa	PS39	0.007	0.596	1.74	0.016	0.006
P. aeruginosa	PS40	0.06	0.557	1.666	0.085	0.004
P. aeruginosa	PS41	0.301	0.64	1.455	0.125	-0.006
P. aeruginosa	PS42	0.014	0.945	1.366	0.035	0.01
P. aeruginosa	PS43	0.008	0.575	1.475	0.142	0.001
P. aeruginosa	PS44	0.001	1.008	1.544	0.033	-0.013
P. aeruginosa	PS45	0.083	0.604	1.632	0.131	-0.033
P. aeruginosa	PS46	0.013	0.586	1.434	0.036	-0.001
P. aeruginosa	PS47	0.014	0.577	1.788	0.017	-0.002
P. aeruginosa	PS48	-0.008	0.901	1.724	0.17	-0.008
P. aeruginosa	PS49	0.072	0.643	1.899	0.079	0.022
P. aeruginosa	PS50	0.091	0.645	1.649	0.137	0.05
P. aeruginosa	PS51	0.012	0.56	1.81	0.176	0.002
P. aeruginosa	PS52	-0.009	0.563	1.675	0.174	-0.015
B. cepacia	LMG 1222	0.314	0.53	1.403	0.322	0.005
B. cepacia	LMG 2161	0.016	0.552	1.344	0.336	-0.008
B. cenocepacia	LMG 16654	0.104	0.695	1.646	0.299	0.014
B. cenocepacia	LMG 16656	0.09	0.607	1.51	0.295	-0.002
B. cenocepacia	LMG 16659	0.005	0.539	1.498	0.288	-0.006
B. cepacia	LMG 17997	0.107	0.647	1.576	0.318	0.009
B. cepacia	LMG 18821	0.119	0.515	1.78	0.135	0.093
B. cenocepacia	LMG 18826	0.161	0.49	1.711	0.159	0.104
B. cenocepacia	LMG 18827	0.004	0.585	1.82	0.006	-0.009
B. cenocepacia	LMG 18828	0.019	0.58	1.818	0.338	0.023
B. cenocepacia	LMG 18829	-0.004	0.619	1.789	0.397	0.005
B. cenocepacia	LMG 18830	0.006	0.663	1.859	0.043	0.001
B. cenocepacia	LMG 18832	0.342	0.599	1.624	0.285	0.03
B. cenocepacia	LMG 18863	0.064	0.615	1.528	0.303	-0.019
B. multivorans	LMG 13010	0.022	0.706	1.598	0.142	0.022
B. multivorans	LMG 16660	0.042	0.66	1.643	0.05	0.004
B. multivorans	LMG 16665	0.04	0.305	1.728	0.265	0.001
B. multivorans	LMG 17588	0.017	0.229	2.126	0.305	-0.007
B. multivorans	LMG 18822	-0.018	0.321	1.925	0.275	-0.001
B. multivorans	LMG 18823	0.021	0.309	1.822	0.264	-0.003
B. multivorans	LMG 18824	-0.021	0.66	1.86	0.316	-0.02
B. multivorans	LMG 18825	-0.025	0.632	1.922	0.334	0.084
B. stabilis	LMG 14086	-0.001	0.706	2.453	0.249	-0.005
B. stabilis	LMG 14294	-0.006	0.698	1.965	0.276	-0.015
B. stabilis	LMG 18870	0.03	0.556	1.79	0.272	0.01
B. stabilis	LMG 18888	-0.003	0.647	2.183	0.268	0.002
B. vietnamiensis	LMG 10929	-0.008	0.416	1.861	0.233	0.018
B. vietnamiensis	LMG 16232	-0.015	0.325	1.969	0.165	-0.03
B. vietnamiensis	LMG 18835	-0.021	0.652	1.807	0.2	-0.009
B. vietnamiensis	LMG 18836	-0.019	0.575	2.168	0.204	0
A. baumannii	ATCC 19606	-0.007	0.752	1.917	-0.003	0.007
A. calcoaceticus	7844	-0.006	0.838	1.883	-0.023	-0.012

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**
A. haemolyticus	12155	-0.017	0.743	1.855	0.035	-0.021
A. johnsonii	10308	0.011	0.67	2.014	-0.146	-0.001
A. lwoffi	5866	0.051	0.788	2.292	-0.074	0
A. lwoffi	5867	0.008	1.041	2.103	0.089	-0.01
A. lwoffi	NCIMB 12456	0.035	0.729	1.547	0.083	0.02
B diminuta	ATCC 11568	0.003	0.763	1.82	0.02	-0.029
B. vesicularis	ATCC 11426	0.001	0.495	1.766	0.011	-0.002
R. pickettii	11149	-0.02	1.631	1.745	0.116	-0.119
C. meningosepticum	ATCC 13253	0.023	0.639	2.113	0.063	-0.015
M. nonliquefaciens	10464	-0.022	0.669	1.972	0.143	-0.017
M. osloensis	10465	-0.019	0.784	2.101	-0.095	-0.001
M. urethralis	11010	0.006	0.828	2.142	0.046	-0.082
O. urethralis	11999	-0.023	0.678	2.294	0.075	-0.014
P. acidovorans	10683	-0.021	0.629	2.002	-0.037	-0.036
P. aeruginosa	6749	-0.012	0.619	2.02	0.075	-0.003
P. aeruginosa	10332	-0.002	0.506	1.817	0.12	0.01
P. alcaligenes	10367	-0.043	0.48	1.795	-0.012	-0.055
P. pseudoalcaligenes	10860	0	0.54	1.438	0.019	-0.022
P. diminuta	8545	-0.033	0.735	1.545	-0.031	0.001
P. fluorescens	10754	0.024	0.582	1.868	0.048	-0.02
P. fluorescens	10392	0.015	0.763	1.809	-0.073	-0.178
P. fluorescens	3756	0.079	0.652	1.807	-0.062	0.03
P. fluorescens	10038	0.01	0.791	1.943	-0.075	-0.004
P. fluorescens	10688	-0.033	0.629	1.833	-0.079	-0.139
P. fluorescens	9428	-0.006	1.057	1.876	-0.052	-0.005
P. fragi	NCIMB 8987	0.054	0.845	-0.011	-0.141	-0.018
P. maltophilia	10257	-0.007	0.512	1.526	0.062	0.007
P. paucimobilis	11030	0.029	0.547	1.739	0.079	-0.018
R. pickettii	11149	0.041	1.408	1.74	0.17	-0.082
P. putida	10936	0.021	0.485	0.549	-0.059	-0.013
P. stutzeri	12262	-0.002	0.532	1.745	0.044	-0.015
P. stutzeri	10475	-0.015	0.55	1.959	0.02	-0.014
P. vesiculare	10900	0.021	0.587	1.982	0.01	-0.001
S. spiritivorum	ATCC 33861	0.013	0.814	2.019	-0.018	0.011
B. ambifaria	11351	-0.006	0.81	2.222	0.279	0.033
B. andropogonis	1279	-0.016	0.606	1.921	0.087	-0.046
B. andropogonis	2126	0.01	0.784	1.877	-0.017	0
B. caryphylli	2155	-0.021	0.625	1.836	0.162	0.005
B. caryphylli	2156	0.002	0.736	1.64	0.104	0.007
B. dolosa	18941	0.006	0.539	1.381	0.007	-0.018
B. dolosa	18942	0.002	0.722	1.881	0.299	-0.02
B. gladioli	11626	0.002	0.753	1.976	0.168	0.017
B. gladioli	18113	-0.019	0.626	1.912	0.252	-0.002
B.gladioli pv. alliicol	2121	-0.004	0.651	2.274	0.051	0.01
B. gladioli pv. alliicol	6877	0.032	0.632	2.003	0.157	-0.122
B.gladioli pv. gladiol	2216	-0.018	0.748	2.019	0.136	0.026
B.gladioli pv. gladiol	6880	-0.009	0.703	2.186	0.213	0.037
B. glumae	1277	-0.021	0.801	2.143	0.278	0.047
B. glumae	2196	0.029	0.85	1.971	0.219	0.016
B. phenazinium	2247	-0.015	0.785	1.22	-0.089	-0.005
B. phenazinium	6868	0.041	0.71	1.601	-0.004	0.001
P. apista	16408	-0.004	0.695	1.64	-0.056	-0.028

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**
P. norimberensis	13019	-0.004	0.465	1.578	-0.169	0.012
P. norimberensis	16603	0.004	0.617	1.787	-0.158	0.018
P. pnomenusa	18087	-0.019	0.591	0.624	-0.082	-0.17
P. pnomenusa	18817	-0.004	0.706	0.652	-0.122	0.058
P. pulmonicola	18107	0.017	0.641	0.612	0.029	0.007
P. sputorum	18100	-0.013	0.597	1.743	-0.138	-0.016
P. sputorum	18819	0.018	0.757	1.218	-0.112	0.013
R. basilensis	18990	0.044	0.799	1.72	0.096	0.044
R. basilensis	19286	0.176	0.849	1.647	-0.009	-0.408
R. campinensis	19282	0.088	1.181	1.628	-0.078	-0.043
R. campinensis	19283	0.048	1.125	1.822	-0.109	-0.002
R. eutropha	1190	0.039	0.695	1.898	0.021	0.012
R. eutropha	1194	0.006	0.767	1.435	0.109	-0.027
R. gilardii	3399	0.005	0.736	1.617	-0.072	-0.002
R. gilardii	3400	0.004	0.67	1.559	-0.023	-0.003
R. mannitolilytica	19090	-0.002	1.121	2.188	0.112	-0.012
R. metallidurans	1195	-0.003	1.245	1.845	0.128	0.008
R. metallidurans	19290	0.054	1.024	1.685	0.151	0.02
R. paucula	3244	-0.021	0.84	2.095	0.165	-0.007
R. paucula	3245	-0.003	0.912	1.88	0.04	-0.02
R. pickettii	5942	0.052	1.602	1.815	0.091	-0.07
R. pickettii	6871	-0.029	1.189	2.054	0.061	-0.029
R. solanacearum	2291	0.013	0.603	1.598	0.148	-0.022
R. solanacearum	2293	0.006	0.443	1.43	-0.024	-0.017
R. taiwanensis	19425	-0.011	0.443	1.747	-0.017	-0.057
S. maltophilia	957	-0.025	0.571	1.941	-0.094	-0.019
S. maltophilia	958	-0.017	0.654	1.892	0.025	-0.065
H_2O		0.005	0.725	-0.203	-0.191	0.005
H_2O		0.003	0.65	-0.204	-0.199	0.013
H_2O		0.001	0.821	-0.2	-0.198	0.002
H_2O		0.002	0.825	-0.194	-0.195	0.006
H_2O		0.006	0.802	-0.196	-0.206	0
E. coli	10418	-0.005	0.627	0.273	-0.037	-0.005
Enterobacter cloacae	11936	-0.028	0.997	0.55	-0.109	-0.04

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**U**	**V**	**W**	**X**	**Y**
P. aeruginosa	2688	9.914	0.021	0.004	0.02	0.254
P. aeruginosa	2702	9.94	0.01	-0.003	-0.001	0.176
P. aeruginosa	2704	0.352	0.015	-0.005	0.001	0.049
P. aeruginosa	2706	0.43	0.008	-0.005	0.001	0.109
P. aeruginosa	2715	0.123	0.057	-0.018	0.014	0.149
P. aeruginosa	2720	0.399	0.069	-0.011	0.008	0.122
P. aeruginosa	2737	-0.001	0.051	-0.005	0.028	0.217
P. aeruginosa	2739	0.022	0.012	-0.013	0.002	0.088
P. aeruginosa	2741	0.527	0.062	0.014	0.025	0.145
P. aeruginosa	2742	0.034	0.105	0.002	0.022	0.263
P. aeruginosa	2749	-0.13	0.081	-0.019	0.061	0.094
P. aeruginosa	2772	2.128	0.035	-0.012	0.012	0.11
P. aeruginosa	2775	1.35	0.08	-0.018	0.032	0.299
P. aeruginosa	2776	0.089	0.024	-0.019	0.017	0.245
P. aeruginosa	2778	0.002	0.098	0.152	0.021	0.263
P. aeruginosa	2779	9.899	0.097	0.247	0.03	0.223
P. aeruginosa	2780	0.392	0.009	-0.005	0.01	0.117
P. aeruginosa	2781	0.537	0.147	0.002	0.028	0.331
P. aeruginosa	2782	2.289	0.034	-0.019	-0.003	0.399
P. aeruginosa	2783	0.068	-0.003	-0.022	-0.002	0.12
P. aeruginosa	PS1	0.763	0.013	0.071	0.005	0.097
P. aeruginosa	PS2	0.493	0.026	-0.035	-0.002	0.111
P. aeruginosa	PS3	0.116	0.017	-0.001	0.006	0.091
P. aeruginosa	PS4	0.014	0.015	-0.01	-0.004	0.061
P. aeruginosa	PS5	0.367	0.108	0.01	0.008	0.254
P. aeruginosa	PS6	0.375	0.036	-0.003	-0.009	0.122
P. aeruginosa	PS7	0.029	0.033	0.002	0.018	0.22
P. aeruginosa	PS8	9.923	0.017	0.01	0.011	0.12
P. aeruginosa	PS9	0.818	0.097	0.01	0.054	0.12
P. aeruginosa	PS10	2.809	0.041	0.003	0.02	0.031
P. aeruginosa	PS11	0.072	0.111	0.018	0.037	0.194
P. aeruginosa	PS12	0.085	0.017	-0.003	-0.004	0.099
P. aeruginosa	PS13	0.027	0.022	0	0.002	0.094
P. aeruginosa	PS14	0.688	0.005	-0.007	-0.01	0.131
P. aeruginosa	PS15	0.006	0.02	-0.002	0.002	0.08
P. aeruginosa	PS16	0.062	0.023	-0.006	0.006	0.108
P. aeruginosa	PS17	0.09	0.107	0.021	0.021	0.326
P. aeruginosa	PS18	0.679	0.022	0.008	0.002	0.231
P. aeruginosa	PS19	0.184	0.02	0.016	0.002	0.181
P. aeruginosa	PS20	0.051	0.023	-0.001	-0.003	0.143
P. aeruginosa	PS21	0.026	0.056	-0.007	0.001	0.133
P. aeruginosa	PS22	1.06	0.085	0.018	0.018	0.188
P. aeruginosa	PS23	0.128	0.022	0.001	0.005	0.11
P. aeruginosa	PS24	0.046	0.015	0.023	0.018	0.246
P. aeruginosa	PS25	0.483	0.052	-0.035	0.012	0.473
P. aeruginosa	PS26	0.189	0.064	0.015	0.015	0.219
P. aeruginosa	PS27	0.117	0.07	-0.01	0.011	0.192
P. aeruginosa	PS28	2.113	0.093	0.012	0.019	0.071
P. aeruginosa	PS29	0.07	0.019	0	0.006	-0.097
P. aeruginosa	PS30	0.144	0.047	0.015	0.013	0.254
P. aeruginosa	PS31	0.487	0.016	0	0.002	0.155
P. aeruginosa	PS32	2.63	0.009	-0.008	0.009	0.188

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**U**	**V**	**W**	**X**	**Y**
P. aeruginosa	PS33	9.925	0.013	-0.01	-0.003	0.168
P. aeruginosa	PS34	9.941	0.057	0.295	0.017	0.133
P. aeruginosa	PS35	9.924	0.097	-0.014	0.008	0.224
P. aeruginosa	PS36	9.917	0.023	0.007	0.008	0.07
P. aeruginosa	PS37	9.907	0.051	-0.025	-0.001	0.121
P. aeruginosa	PS38	0.363	0.013	-0.016	-0.009	0.108
P. aeruginosa	PS39	0.296	0.009	-0.001	0.002	0.073
P. aeruginosa	PS40	0.476	0.052	-0.006	0.015	0.07
P. aeruginosa	PS41	0.846	0.012	-0.01	-0.006	0.195
P. aeruginosa	PS42	0.105	0.005	-0.006	-0.007	0.148
P. aeruginosa	PS43	0	0.001	0.01	0.016	0.241
P. aeruginosa	PS44	0.085	0.003	-0.012	-0.005	0.171
P. aeruginosa	PS45	0.052	0.111	-0.021	0.014	0.196
P. aeruginosa	PS46	0.035	0.021	-0.007	0.004	0.124
P. aeruginosa	PS47	0.072	0.027	-0.001	0.011	0.144
P. aeruginosa	PS48	0.024	0.059	0.075	0.028	0.147
P. aeruginosa	PS49	0.081	0.059	0.076	0.017	0.102
P. aeruginosa	PS50	0.093	0.021	0.025	0.011	0.093
P. aeruginosa	PS51	0.02	0.01	-0.008	-0.03	0.143
P. aeruginosa	PS52	0.134	0.021	-0.031	-0.004	0.102
B. cepacia	LMG 1222	0.031	0.136	-0.014	-0.01	0.113
B. cepacia	LMG 2161	0.089	0.054	-0.012	-0.007	0.075
B. cenocepacia	LMG 16654	0.085	0.196	-0.02	0.005	0.122
B. cenocepacia	LMG 16656	-0.021	0.174	-0.007	0.018	0.066
B. cenocepacia	LMG 16659	0.015	0.073	-0.01	-0.001	0.062
B. cepacia	LMG 17997	0.194	0.181	0.041	0.102	0.071
B. cepacia	LMG 18821	0.117	0.119	-0.007	0.031	0.057
B. cenocepacia	LMG 18826	0.143	0.123	-0.004	0.039	0.048
B. cenocepacia	LMG 18827	0.016	0.025	-0.002	0.004	0.009
B. cenocepacia	LMG 18828	0.067	0.109	0.016	-0.001	0.054
B. cenocepacia	LMG 18829	0.086	0.186	-0.002	0.005	0.089
B. cenocepacia	LMG 18830	0.164	0.022	-0.004	0.017	0.03
B. cenocepacia	LMG 18832	1.651	0.212	0.023	0.044	0.078
B. cenocepacia	LMG 18863	0.007	0.137	0.046	0.088	0.071
B. multivorans	LMG 13010	9.914	0.081	0.005	0.031	0.057
B. multivorans	LMG 16660	0.061	0.018	-0.005	-0.004	0.04
B. multivorans	LMG 16665	0.037	0.064	-0.011	0.012	0.043
B. multivorans	LMG 17588	-0.033	0.02	-0.012	0.021	0.037
B. multivorans	LMG 18822	-0.029	0.019	-0.003	-0.014	0.04
B. multivorans	LMG 18823	-0.018	0.031	-0.001	0.004	0.029
B. multivorans	LMG 18824	-0.051	0.049	-0.019	-0.002	0.011
B. multivorans	LMG 18825	-0.041	0.028	-0.017	-0.016	0.006
B. stabilis	LMG 14086	0.055	0.031	-0.009	0.003	0.011
B. stabilis	LMG 14294	0.011	0.031	-0.021	-0.009	0.042
B. stabilis	LMG 18870	9.908	0.093	0.063	0.08	0.001
B. stabilis	LMG 18888	9.938	0.033	-0.003	0.176	0.089
B. vietnamiensis	LMG 10929	0.353	0.086	0.008	0.001	0.062
B. vietnamiensis	LMG 16232	0.383	0.071	-0.005	0.013	0.016
B. vietnamiensis	LMG 18835	0.133	0.037	-0.01	0.004	0.001
B. vietnamiensis	LMG 18836	0.372	0.161	0.036	0.017	0.026
A. baumannii	ATCC 19606	0.001	0.015	-0.004	-0.005	0.026
A. calcoaceticus	7844	0.01	0.016	-0.007	-0.012	0.095

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**U**	**V**	**W**	**X**	**Y**
A. haemolyticus	12155	0.582	0.008	-0.022	-0.008	0.065
A. johnsonii	10308	0.048	0.03	0.027	0.006	-0.008
A. lwoffi	5866	0.01	0	-0.005	-0.001	-0.015
A. lwoffi	5867	2.055	0.017	0.011	0.013	0.024
A. lwoffi	NCIMB 12456	1.304	0.072	0.062	0.027	0.038
B diminuta	ATCC 11568	0.073	0.143	0.037	0.016	1.49
B. vesicularis	ATCC 11426	-0.018	0.031	-0.006	-0.008	1.595
R. pickettii	11149	0.354	0.159	0.148	0.08	0
C. meningosepticum	ATCC 13253	0.501	0.616	1.168	0.362	1.043
M. nonliquefaciens	10464	2.264	0.022	0.148	0.003	0.194
M. osloensis	10465	0.099	0.093	0.141	0.005	0.057
M. urethralis	11010	0.456	0.027	0.288	0.035	0.019
O. urethralis	11999	0.51	0.165	0.375	0.006	0.39
P. acidovorans	10683	0.021	-0.003	0.089	-0.031	0.02
P. aeruginosa	6749	-0.009	0	-0.012	-0.01	0.042
P. aeruginosa	10332	0.357	0.011	0.103	-0.001	0.052
P. alcaligenes	10367	0.307	-0.026	-0.05	-0.055	0.037
P. pseudoalcaligenes	10860	0.027	0.021	0.024	-0.003	0.074
P. diminuta	8545	0.828	0.116	0.003	-0.003	1.667
P. fluorescens	10754	2.778	0.239	0.236	0.063	0.529
P. fluorescens	10392	0.092	0.064	-0.022	0.007	0.426
P. fluorescens	3756	0.084	0.087	0.168	0.05	0.623
P. fluorescens	10038	0.035	0.039	0.008	0.018	0.227
P. fluorescens	10688	0.471	0.033	-0.009	-0.01	0.129
P. fluorescens	9428	-0.008	0.001	0.002	-0.014	0.338
P. fragi	NCIMB 8987	0.039	0.124	0.002	0.049	0.059
P. maltophilia	10257	0.096	0.155	0.026	0.014	0.494
P. paucimobilis	11030	0.65	0.004	0.073	0.64	0.333
R. pickettii	11149	0.188	0.088	0.139	-0.007	0.048
P. putida	10936	0.041	0.06	0.018	0.009	0.106
P. stutzeri	12262	0.038	0.005	0.319	-0.008	0.424
P. stutzeri	10475	1.052	0.016	0.214	0.001	0.324
P. vesiculare	10900	0.123	0.117	0.01	0.022	1.537
S. spiritivorum	ATCC 33861	0.058	0.052	0.284	0.399	1.363
B. ambifaria	11351	0.522	0.138	0.044	0.026	0.186
B. andropogonis	1279	0.173	0.338	0.625	-0.005	1.211
B. andropogonis	2126	0.102	0.153	0.39	0.241	1.203
B. caryphylli	2155	2.027	0.038	0.215	0.009	0.032
B. caryphylli	2156	0.081	0.031	0.021	-0.01	0.169
B. dolosa	18941	0.127	0.259	0.006	0.122	0.261
B. dolosa	18942	0.433	0.31	0.26	0.229	0.465
B. gladioli	11626	2.57	0.062	-0.009	-0.017	0.025
B. gladioli	18113	9.892	0.065	0.283	-0.002	0.042
B.gladioli pv. alliicola	2121	9.889	0.05	0.001	-0.007	0.037
B. gladioli pv. alliicola	6877	9.896	0.09	0.032	0.005	0.069
B.gladioli pv. gladioli	2216	9.924	0.024	-0.011	-0.008	0.034
B.gladioli pv. gladioli	6880	9.918	0.024	-0.001	-0.013	0.035
B. glumae	1277	0.34	0.031	0.007	0.009	0.047
B. glumae	2196	0.238	0.065	0.035	-0.016	0.057
B. phenazinium	2247	0.459	0.033	-0.01	0.049	0.244
B. phenazinium	6868	0.872	0.01	0.008	0	0.019
P. apista	16408	0.014	0.043	-0.001	-0.052	0.021

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**U**	**V**	**W**	**X**	**Y**
P. norimberensis	13019	-0.001	0.015	-0.017	-0.013	1.03
P. norimberensis	16603	0.04	0.021	-0.023	-0.009	0.084
P. pnomenusa	18087	0.054	0.193	-0.01	0.014	0.017
P. pnomenusa	18817	0.006	-0.001	-0.02	-0.013	0.012
P. pulmonicola	18107	0.068	0.083	-0.003	0	0.011
P. sputorum	18100	0.01	0.018	0.081	-0.018	-0.001
P. sputorum	18819	0.124	0.054	0.033	0.016	0.035
R. basilensis	18990	0.092	0.058	0.008	0.026	0.034
R. basilensis	19286	0.101	0.069	0.039	0.032	0.004
R. campinensis	19282	0.115	0.031	0.051	-0.004	0
R. campinensis	19283	0.016	0.054	0.011	-0.019	0.045
R. eutropha	1190	0.087	0.118	0.169	0.454	0.557
R. eutropha	1194	0.058	0.014	-0.022	-0.003	0.045
R. gilardii	3399	-0.001	0.052	-0.017	-0.014	0.021
R. gilardii	3400	0.002	0.106	-0.007	0.016	0.01
R. mannitolilytica	19090	0.163	0.243	0.484	0.011	1.093
R. metallidurans	1195	0.131	0.036	0.014	0.034	0.029
R. metallidurans	19290	0.229	0.07	-0.001	0.056	0.055
R. paucula	3244	0.008	0.048	-0.001	-0.006	0.119
R. paucula	3245	0.078	0.027	-0.043	-0.003	0.021
R. pickettii	5942	0.076	0.003	-0.05	-0.003	0.141
R. pickettii	6871	0.141	0.011	-0.015	0.016	-0.009
R. solanacearum	2291	1.636	0.045	0.044	-0.014	0.062
R. solanacearum	2293	0.072	0.05	-0.016	0.042	0.132
R. taiwanensis	19425	9.828	0.478	0.701	0.046	0.44
S. maltophilia	957	0.007	0.137	-0.005	0.086	1.184
S. maltophilia	958	0.023	0.29	0.01	0.091	0.576
H_2O		0.002	0.012	0	0	0.027
H_2O		0	-0.003	0.003	0.001	0.017
H_2O		0	0.003	0.002	-0.001	0.022
H_2O		0	0.004	0.001	0.001	0.02
H_2O		0.003	0	0.002	-0.001	0.016
E. coli	10418	0.018	0.119	0.007	0.006	0.168
Enterobacter cloacae	11936	-0.024	0.117	-0.019	0.221	0.03

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**Z**	**AB**	**AC**	**AD**	**AE**
P. aeruginosa	2688	0.306	0.006	-0.003	-0.003	2.573
P. aeruginosa	2702	0.335	-0.001	-0.008	-0.011	2.676
P. aeruginosa	2704	0.044	0.038	0.019	-0.005	2.403
P. aeruginosa	2706	0.305	0.006	-0.007	-0.009	2.481
P. aeruginosa	2715	0.363	0.029	-0.01	0.01	2.477
P. aeruginosa	2720	0.174	0	-0.014	0.006	2.402
P. aeruginosa	2737	0.178	0.026	-0.002	0.014	2.589
P. aeruginosa	2739	0.145	0.004	-0.009	-0.012	2.592
P. aeruginosa	2741	0.131	0.036	-0.014	-0.018	2.557
P. aeruginosa	2742	0.539	0.026	-0.011	-0.017	2.617
P. aeruginosa	2749	0.19	0.018	-0.014	0.01	2.497
P. aeruginosa	2772	0.317	-0.012	-0.022	0.103	2.572
P. aeruginosa	2775	0.292	0.004	-0.055	-0.024	2.381
P. aeruginosa	2776	0.249	0.018	0	-0.005	2.408
P. aeruginosa	2778	0.047	0.017	0.06	0.021	2.345
P. aeruginosa	2779	0.828	0.024	0.01	0.003	2.368
P. aeruginosa	2780	0.134	0.009	0.008	-0.005	2.33
P. aeruginosa	2781	0.415	0.09	0.064	0.009	2.521
P. aeruginosa	2782	0.635	-0.007	-0.018	-0.032	2.454
P. aeruginosa	2783	0.316	-0.01	-0.004	-0.008	2.476
P. aeruginosa	PS1	0.132	-0.005	-0.011	-0.013	2.561
P. aeruginosa	PS2	0.21	-0.002	-0.216	-0.033	2.578
P. aeruginosa	PS3	0.268	0.001	-0.004	-0.003	2.624
P. aeruginosa	PS4	0.929	-0.004	-0.011	-0.007	2.536
P. aeruginosa	PS5	0.687	0.043	0.004	0.008	2.515
P. aeruginosa	PS6	0.115	0.001	-0.002	-0.001	2.549
P. aeruginosa	PS7	0.144	0.019	0.003	-0.007	2.343
P. aeruginosa	PS8	0.243	-0.003	-0.005	-0.006	2.47
P. aeruginosa	PS9	0.242	0.009	0.009	0.003	2.377
P. aeruginosa	PS10	0.019	0.001	0.002	0.001	0.584
P. aeruginosa	PS11	0.772	-0.166	0.052	0.019	2.558
P. aeruginosa	PS12	0.18	-0.007	-0.012	-0.012	2.612
P. aeruginosa	PS13	0.219	-0.007	-0.012	-0.014	2.53
P. aeruginosa	PS14	0.179	-0.005	-0.008	0.023	2.552
P. aeruginosa	PS15	0.125	-0.007	-0.011	-0.015	2.481
P. aeruginosa	PS16	0.449	-0.017	-0.019	-0.03	9.753
P. aeruginosa	PS17	0.335	0.027	0.009	0	2.327
P. aeruginosa	PS18	0.287	-0.001	-0.008	-0.008	9.75
P. aeruginosa	PS19	0.347	-0.012	-0.008	-0.011	2.264
P. aeruginosa	PS20	0.228	0.027	0.009	0.016	2.417
P. aeruginosa	PS21	0.123	-0.001	-0.001	-0.015	2.404
P. aeruginosa	PS22	0.371	0	0.001	-0.018	2.51
P. aeruginosa	PS23	0.058	0.01	-0.008	0.003	2.424
P. aeruginosa	PS24	0.153	-0.008	-0.004	-0.005	2.345
P. aeruginosa	PS25	0.745	-0.053	-0.043	-0.062	2.545
P. aeruginosa	PS26	0.738	0.023	0.022	0.01	2.507
P. aeruginosa	PS27	0.343	0.007	-0.009	-0.018	2.532
P. aeruginosa	PS28	0.35	0.027	0.012	0.009	2.548
P. aeruginosa	PS29	0.13	-0.001	-0.002	-0.001	2.159
P. aeruginosa	PS30	0.272	0.017	-0.01	0.013	2.131
P. aeruginosa	PS31	0.021	0.001	-0.006	-0.004	2.178
P. aeruginosa	PS32	0.081	0	0.001	-0.003	2.301

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**Z**	**AB**	**AC**	**AD**	**AE**
P. aeruginosa	PS33	0.166	-0.014	-0.01	-0.017	2.391
P. aeruginosa	PS34	0.015	0.007	-0.006	-0.005	2.244
P. aeruginosa	PS35	0.135	0.045	-0.005	-0.021	2.374
P. aeruginosa	PS36	0.056	-0.002	-0.004	-0.008	2.506
P. aeruginosa	PS37	0.04	-0.004	-0.013	-0.019	2.395
P. aeruginosa	PS38	0.143	-0.019	-0.019	-0.015	2.511
P. aeruginosa	PS39	0.126	-0.003	-0.012	-0.01	2.558
P. aeruginosa	PS40	0.045	0.026	-0.009	-0.015	2.349
P. aeruginosa	PS41	0.235	0.014	-0.002	0	2.267
P. aeruginosa	PS42	0.15	-0.008	-0.01	-0.018	2.215
P. aeruginosa	PS43	0.387	0.033	-0.013	0.001	2.319
P. aeruginosa	PS44	0.098	-0.019	-0.008	-0.011	2.311
P. aeruginosa	PS45	0.437	0.09	0.029	0.022	2.366
P. aeruginosa	PS46	0.069	0.005	0.004	-0.01	2.491
P. aeruginosa	PS47	0.088	0.011	0.002	-0.008	2.446
P. aeruginosa	PS48	0.247	0.009	0.006	-0.002	9.761
P. aeruginosa	PS49	0.175	0.019	0.014	-0.008	2.499
P. aeruginosa	PS50	0.415	-0.013	-0.01	-0.019	2.418
P. aeruginosa	PS51	0.187	0.003	-0.009	-0.006	2.588
P. aeruginosa	PS52	0.205	0.002	-0.02	-0.015	2.44
B. cepacia	LMG 1222	0.112	0.089	0.034	0.065	2.634
B. cepacia	LMG 2161	0.044	0.057	-0.004	0.005	2.173
B. cenocepacia	LMG 16654	0.129	0.079	0.048	0.037	2.48
B. cenocepacia	LMG 16656	0.12	0.044	0.043	0.034	2.56
B. cenocepacia	LMG 16659	0.046	-0.013	-0.02	-0.03	2.36
B. cepacia	LMG 17997	0.204	0.032	0.037	0.031	2.587
B. cepacia	LMG 18821	0.052	0.025	0.031	0.019	2.513
B. cenocepacia	LMG 18826	0.057	0.064	0.024	0.02	2.454
B. cenocepacia	LMG 18827	0.003	-0.013	-0.013	-0.023	2.566
B. cenocepacia	LMG 18828	0.257	0.002	0.039	-0.004	2.712
B. cenocepacia	LMG 18829	0.209	0.049	0.026	0.026	2.598
B. cenocepacia	LMG 18830	0.037	0.008	0.025	0.006	9.759
B. cenocepacia	LMG 18832	0.136	0.12	0.065	0.056	2.47
B. cenocepacia	LMG 18863	0.043	0.119	0.059	0.084	2.477
B. multivorans	LMG 13010	0.058	0.085	0.031	0.021	2.554
B. multivorans	LMG 16660	0.066	0.049	0.031	0.003	2.626
B. multivorans	LMG 16665	0.088	0.024	0.021	0.011	2.505
B. multivorans	LMG 17588	0.035	0.001	-0.002	-0.012	2.686
B. multivorans	LMG 18822	0.016	-0.018	-0.019	-0.02	2.603
B. multivorans	LMG 18823	0.011	-0.013	-0.011	-0.013	2.711
B. multivorans	LMG 18824	0.058	0.008	-0.01	-0.007	2.573
B. multivorans	LMG 18825	0.013	-0.024	-0.026	-0.03	2.52
B. stabilis	LMG 14086	0.031	-0.015	-0.011	-0.027	2.578
B. stabilis	LMG 14294	0.008	-0.015	-0.018	-0.03	2.496
B. stabilis	LMG 18870	0.01	0.008	0.002	-0.009	2.423
B. stabilis	LMG 18888	0.154	-0.072	-0.01	-0.018	2.113
B. vietnamiensis	LMG 10929	1.452	0.009	0.051	0.079	2.275
B. vietnamiensis	LMG 16232	0.014	-0.019	-0.017	-0.002	2.274
B. vietnamiensis	LMG 18835	0.035	-0.032	-0.021	-0.02	9.71
B. vietnamiensis	LMG 18836	0.016	-0.017	0.008	-0.004	9.748
A. baumannii	ATCC 1960€	-0.01	-0.012	-0.012	0.165	2.752
A. calcoaceticus	7844	-0.008	-0.018	-0.016	0.159	2.654

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**Z**	**AB**	**AC**	**AD**	**AE**
A. haemolyticus	12155	-0.002	-0.012	-0.022	0.08	2.655
A. johnsonii	10308	0.011	-0.021	0.001	-0.022	9.75
A. lwoffi	5866	-0.025	-0.059	0.006	-0.017	2.675
A. lwoffi	5867	0.024	-0.005	-0.008	0.081	2.72
A. lwoffi	NCIMB 1245(	0.021	0.041	0.182	0.052	1.56
B diminuta	ATCC 1156E	0.016	0.008	-0.003	0.019	2.017
B. vesicularis	ATCC 1142E	0.002	0.455	0.022	-0.006	1.734
R. pickettii	11149	0.108	0.534	0.015	0.021	9.708
C. meningosepticum	ATCC 13253	0.637	1.209	0.183	0.002	9.737
M. nonliquefaciens	10464	0.58	0.081	-0.013	-0.012	2.678
M. osloensis	10465	0.262	0.161	-0.004	0.003	2.568
M. urethralis	11010	0.074	0.005	0.01	0	0.616
O. urethralis	11999	1.197	1.05	0.006	-0.06	9.727
P. acidovorans	10683	0.508	0.007	0	-0.004	2.544
P. aeruginosa	6749	0.195	0.084	-0.007	-0.012	9.769
P. aeruginosa	10332	0.211	0.001	-0.012	0.005	2.005
P. alcaligenes	10367	0.047	1.079	0.195	-0.061	2.156
P. pseudoalcaligenes	10860	-0.008	-0.02	-0.01	-0.032	2.061
P. diminuta	8545	0.06	0.42	-0.019	-0.022	2.07
P. fluorescens	10754	1.723	0.751	0.055	-0.006	2.627
P. fluorescens	10392	0.148	0.621	-0.004	-0.01	2.375
P. fluorescens	3756	0.407	0.444	0.016	-0.025	2.574
P. fluorescens	10038	0.07	0.007	0.016	-0.002	2.628
P. fluorescens	10688	0.166	0.007	0.023	-0.024	2.563
P. fluorescens	9428	0.003	-0.016	-0.019	-0.019	2.613
P. fragi	NCIMB 8987	1.096	0.003	0	-0.001	1.295
P. maltophilia	10257	0.505	2.85	0.138	-0.007	2.07
P. paucimobilis	11030	0.157	0.154	0.098	0.064	1.613
R. pickettii	11149	0.411	0.421	0.077	0.026	9.654
P. putida	10936	0.113	0.039	0.029	0.021	2.344
P. stutzeri	12262	0.057	-0.018	-0.008	-0.024	2.466
P. stutzeri	10475	0.054	9.896	-0.016	-0.02	2.684
P. vesiculare	10900	0.188	0.778	0.075	0.031	2.566
S. spiritivorum	ATCC 33861	0.109	1.821	1.541	0.158	9.682
B. ambifaria	11351	0.052	0.307	0.294	0.018	9.754
B. andropogonis	1279	1.177	9.869	0.104	-0.034	9.729
B. andropogonis	2126	1.01	1.817	1.232	0.089	9.677
B. caryphylli	2155	0.014	0.015	0.057	-0.024	9.711
B. caryphylli	2156	0.371	0.24	0.063	-0.001	2.384
B. dolosa	18941	0.463	0.423	0.384	0.017	2.439
B. dolosa	18942	0.471	1.327	0.253	0.009	2.613
B. gladioli	11626	0.02	0.932	0	-0.001	9.708
B. gladioli	18113	0.013	-0.008	-0.011	-0.016	9.736
B.gladioli pv. alliicola	2121	0.014	-0.007	-0.006	-0.016	9.753
B. gladioli pv. alliicola	6877	0.013	0.057	0.051	-0.004	9.749
B.gladioli pv. gladioli	2216	0.023	-0.012	0	-0.011	9.721
B.gladioli pv. gladioli	6880	0.008	0.505	-0.007	-0.017	9.745
B. glumae	1277	0.005	0.275	-0.013	-0.036	9.724
B. glumae	2196	-0.012	0.002	-0.001	-0.01	9.721
B. phenazinium	2247	0.631	1.391	-0.013	-0.043	2.587
B. phenazinium	6868	0.003	-0.006	-0.014	-0.011	2.125
P. apista	16408	0.09	0.015	0.007	0.002	2.642

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**Z**	**AB**	**AC**	**AD**	**AE**
P. norimberensis	13019	0.013	1.283	0.239	0.018	2.684
P. norimberensis	16603	0.17	0.117	0.076	-0.013	9.737
P. pnomenusa	18087	0.146	0	-0.008	-0.013	2.466
P. pnomenusa	18817	0.081	0.003	0.008	0.002	2.173
P. pulmonicola	18107	0.288	0.012	0.008	-0.011	1.986
P. sputorum	18100	0.043	-0.008	-0.017	-0.021	9.76
P. sputorum	18819	0.086	0.035	0.021	-0.004	2.557
R. basilensis	18990	0.034	0.005	0.011	-0.019	9.727
R. basilensis	19286	0.064	0.038	0.03	0.01	9.725
R. campinensis	19282	-0.013	-0.015	0.008	0.001	9.72
R. campinensis	19283	0.004	0.08	0.039	0.016	2.525
R. eutropha	1190	0.692	1.433	0.774	0.034	2.416
R. eutropha	1194	-0.037	0.009	0.034	0.022	2.485
R. gilardii	3399	0.009	0.007	-0.003	-0.001	2.076
R. gilardii	3400	-0.004	0.007	-0.007	-0.012	2.507
R. mannitolilytica	19090	1.391	1.513	0.121	0.016	2.007
R. metallidurans	1195	-0.01	0.039	0.02	0.021	9.743
R. metallidurans	19290	-0.012	0.047	0.035	0.012	9.744
R. paucula	3244	-0.028	-0.002	0.009	-0.008	9.706
R. paucula	3245	0.021	0.021	0.086	-0.017	9.703
R. pickettii	5942	0.2	0.059	0.206	0.041	9.658
R. pickettii	6871	0.085	-0.012	0.015	-0.021	2.697
R. solanacearum	2291	0.052	0.007	0.066	0.012	2.223
R. solanacearum	2293	0.112	2.035	0.023	-0.001	2.638
R. taiwanensis	19425	0.827	0.991	0.017	0.001	2.713
S. maltophilia	957	0.695	9.87	0.265	-0.001	2.631
S. maltophilia	958	0.556	9.915	0.105	-0.018	9.768
H2O		0.023	0.006	0.004	0.008	0.976
H2O		0.012	0.014	0.018	0.012	1.019
H2O		0.013	0.005	0	0.002	1.068
H2O		0.018	0.004	0.002	0.004	1.084
H2O		0.027	0.016	0.006	0.023	1.033
E. coli	10418	0.023	0.072	-0.015	-0.021	1.014
Enterobacter cloacae	11936	0.202	-0.004	0.016	-0.019	1.138

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**AQ**	**AR**	**AS**	**AT**	**AU**
P. aeruginosa	2688	0.136	1.568	0.034	0.019	0.216
P. aeruginosa	2702	0.214	0.171	0.785	0.497	0.281
P. aeruginosa	2704	0.07	0.222	0.504	0.166	0.168
P. aeruginosa	2706	0.755	0.561	0.282	0.089	0.405
P. aeruginosa	2715	0.307	0.602	0.515	-0.007	0.053
P. aeruginosa	2720	0.087	0.175	0.635	0.005	0.015
P. aeruginosa	2737	0.128	0.398	0.351	-0.018	0.016
P. aeruginosa	2739	0.121	0.057	0.484	-0.037	0.002
P. aeruginosa	2741	0.015	0.137	0.666	0.025	0.143
P. aeruginosa	2742	0.13	0.621	0.711	-0.014	0.001
P. aeruginosa	2749	0.196	0.613	0.572	-0.011	0.012
P. aeruginosa	2772	0.212	0.38	0.93	0.004	0.025
P. aeruginosa	2775	0.118	0.089	0.988	-0.006	-0.012
P. aeruginosa	2776	0.277	0.265	0.438	-0.014	-0.007
P. aeruginosa	2778	0.084	0.36	0.625	0.036	0.016
P. aeruginosa	2779	0.369	0.114	0.39	-0.002	0.009
P. aeruginosa	2780	0.145	0.957	0.633	-0.002	-0.001
P. aeruginosa	2781	0.114	0.276	0.276	0.007	0.031
P. aeruginosa	2782	0.178	0.153	0.577	-0.015	0.017
P. aeruginosa	2783	0.414	0.902	0.254	-0.008	0.023
P. aeruginosa	PS1	0.316	1.917	0.261	-0.014	0.011
P. aeruginosa	PS2	0.19	1.238	0.184	-0.022	-0.002
P. aeruginosa	PS3	0.217	0.211	0.104	0.297	0.317
P. aeruginosa	PS4	0.214	0.231	0.287	0.504	0.197
P. aeruginosa	PS5	1.028	1.262	1.048	-0.011	0.001
P. aeruginosa	PS6	0.103	0.3	0.809	0.485	0.297
P. aeruginosa	PS7	0.038	0.245	0.747	0.106	0.162
P. aeruginosa	PS8	0.067	0.07	0.417	0.411	0.15
P. aeruginosa	PS9	0.5	0.189	0.302	0.649	0.357
P. aeruginosa	PS10	0.111	0.15	0.724	0.054	0.105
P. aeruginosa	PS11	0.612	1.99	0.464	0.002	0.033
P. aeruginosa	PS12	0.076	0.167	1.231	0.638	0.216
P. aeruginosa	PS13	0.042	0.5	0.339	-0.009	0.018
P. aeruginosa	PS14	0.176	0.758	0.317	-0.015	0.02
P. aeruginosa	PS15	0.208	1.275	0.136	-0.002	0.019
P. aeruginosa	PS16	0.189	1.21	0.808	-0.005	0.031
P. aeruginosa	PS17	0.607	1.245	0.61	-0.009	2.341
P. aeruginosa	PS18	0.039	0.092	0.522	0.217	0.156
P. aeruginosa	PS19	0.114	1.352	0.556	0.061	0.863
P. aeruginosa	PS20	0.22	0.608	0.41	0.005	1.228
P. aeruginosa	PS21	0.041	0.086	0.367	0	1.188
P. aeruginosa	PS22	0.056	0.073	0.392	0.215	0.169
P. aeruginosa	PS23	0.08	0.399	0.31	0	0.99
P. aeruginosa	PS24	0.018	0.333	0.473	-0.001	0.024
P. aeruginosa	PS25	0.653	1.683	-0.045	-0.014	2.644
P. aeruginosa	PS26	0.094	0.537	0.319	0.046	0.196
P. aeruginosa	PS27	0.407	0.221	0.088	0.113	0.44
P. aeruginosa	PS28	0.133	0.104	0.436	0.143	0.211
P. aeruginosa	PS29	0.034	0.517	1.021	-0.004	0.685
P. aeruginosa	PS30	0.29	1.585	0.544	0.016	0.994
P. aeruginosa	PS31	0.072	0.711	0.368	-0.005	0.673
P. aeruginosa	PS32	0.043	0.232	0.45	0.009	0.936

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**AQ**	**AR**	**AS**	**AT**	**AU**
P. aeruginosa	PS33	0.228	1.772	0.286	-0.017	0.946
P. aeruginosa	PS34	0.005	0.932	0.113	0.005	1.956
P. aeruginosa	PS35	0.128	0.395	0.21	-0.002	2.855
P. aeruginosa	PS36	0.034	0.126	0.343	0.199	0.137
P. aeruginosa	PS37	0.529	1.417	0.21	-0.019	2.56
P. aeruginosa	PS38	0.01	0.348	0.662	0.022	0.092
P. aeruginosa	PS39	0.12	1.322	0.043	0.015	0.13
P. aeruginosa	PS40	0.166	0.614	0.241	0.135	0.285
P. aeruginosa	PS41	0.352	0.21	0.974	-0.002	2.411
P. aeruginosa	PS42	0.109	0.592	0.346	0.056	0.389
P. aeruginosa	PS43	0.198	0.9	0.558	0.049	0.43
P. aeruginosa	PS44	0.061	0.065	0.082	-0.005	2.07
P. aeruginosa	PS45	0.543	1.713	0.527	0.009	2.122
P. aeruginosa	PS46	0.06	0.656	0.49	0.001	0.693
P. aeruginosa	PS47	0.053	0.385	0.75	-0.005	0.473
P. aeruginosa	PS48	0.223	0.098	0.472	-0.012	0.608
P. aeruginosa	PS49	0.12	0.634	0.603	0.121	0.361
P. aeruginosa	PS50	0.296	2.027	0.38	-0.034	2.58
P. aeruginosa	PS51	0.097	0.921	0.442	-0.015	0.733
P. aeruginosa	PS52	0.369	1.702	0.388	-0.024	2.364
B. cepacia	LMG 1222	0.021	0.3	0.992	0.003	0.188
B. cepacia	LMG 2161	-0.006	0.01	0.886	-0.016	0.26
B. cenocepacia	LMG 16654	0.009	0.097	0.863	-0.015	0.037
B. cenocepacia	LMG 16656	0.008	0.128	0.601	-0.011	0.119
B. cenocepacia	LMG 16659	-0.003	0.011	0.727	-0.011	0.15
B. cepacia	LMG 17997	0.058	0.287	0.951	-0.012	0.449
B. cepacia	LMG 18821	-0.013	0.009	0.661	-0.032	1.456
B. cenocepacia	LMG 18826	0.001	0.029	0.08	-0.019	0.585
B. cenocepacia	LMG 18827	0.005	0.523	0.652	-0.009	0.502
B. cenocepacia	LMG 18828	0.075	0.146	0.44	0.007	0.1
B. cenocepacia	LMG 18829	0	0.262	0.745	-0.031	0.354
B. cenocepacia	LMG 18830	0.036	0.294	0.9	0.021	0.071
B. cenocepacia	LMG 18832	1.452	0.613	0.885	0	1.685
B. cenocepacia	LMG 18863	0.098	0.349	0.872	0.104	1.282
B. multivorans	LMG 13010	0.04	0.413	0.8	0.006	0.247
B. multivorans	LMG 16660	0.007	0.291	0.852	-0.011	0.265
B. multivorans	LMG 16665	-0.005	0.297	0.61	-0.02	0.216
B. multivorans	LMG 17588	0.003	0.387	0.478	-0.011	0.402
B. multivorans	LMG 18822	0.004	0.528	0.234	-0.019	0.373
B. multivorans	LMG 18823	0	0.219	0.738	-0.073	0.25
B. multivorans	LMG 18824	-0.005	0.057	0.679	-0.014	0.599
B. multivorans	LMG 18825	-0.006	0.148	0.797	-0.022	1.695
B. stabilis	LMG 14086	0	0.02	0.994	-0.013	0.311
B. stabilis	LMG 14294	-0.005	0.009	0.981	-0.018	0.553
B. stabilis	LMG 18870	-0.004	0.083	0.171	-0.009	-0.001
B. stabilis	LMG 18888	0.013	0.034	0.371	-0.006	0
B. vietnamiensis	LMG 10929	-0.003	0.081	0.588	0.028	0.058
B. vietnamiensis	LMG 16232	0.037	0.22	0.225	-0.017	0.033
B. vietnamiensis	LMG 18835	-0.008	0.086	0.504	-0.021	-0.012
B. vietnamiensis	LMG 18836	0.003	0.111	0.481	-0.011	0.011
A. baumannii	ATCC 1960€	-0.008	0.05	0.299	0.03	-0.006
A. calcoaceticus	7844	0.025	0.288	0.096	0.056	0.027

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**AQ**	**AR**	**AS**	**AT**	**AU**
A. haemolyticus	12155	0.002	0.226	0.146	0.016	0.028
A. johnsonii	10308	-0.019	0.226	0.424	0.04	-0.025
A. lwoffi	5866	0.006	0.042	0.21	0.015	-0.002
A. lwoffi	5867	0.105	0.705	0.643	0.033	-0.011
A. lwoffi	NCIMB 1245(	-0.002	0.148	-0.087	-0.016	0.339
B diminuta	ATCC 1156E	0.028	0.912	0.103	0.221	1.154
B. vesicularis	ATCC 1142E	0.008	9.883	0.716	0.606	2.61
R. pickettii	11149	0.085	0.283	0.541	0.314	2.628
C. meningosepticum	ATCC 1325:	0.787	9.882	1.247	0.544	0.508
M. nonliquefaciens	10464	0.611	2.538	0.103	-0.027	0
M. osloensis	10465	0.06	1.609	0.593	-0.005	0.681
M. urethralis	11010	0.142	0.088	0.343	-0.008	-0.012
O. urethralis	11999	1.856	2.837	0.8	0.03	0.132
P. acidovorans	10683	0.373	0.096	0.476	-0.01	0.008
P. aeruginosa	6749	0.198	0.042	0.458	-0.015	-0.004
P. aeruginosa	10332	0.477	2.399	0.625	-0.013	0.008
P. alcaligenes	10367	-0.011	1.971	-0.025	-0.036	-0.015
P. pseudoalcaligenes	10860	-0.015	0.429	0.335	0.043	-0.008
P. diminuta	8545	0.06	2.161	0.325	1.131	9.901
P. fluorescens	10754	1.326	9.9	0.512	0.26	1.391
P. fluorescens	10392	0.152	0.076	0.196	0.016	0.244
P. fluorescens	3756	0.054	1.993	0.188	-0.012	0.025
P. fluorescens	10038	0.267	0.202	0.18	0.003	0.084
P. fluorescens	10688	0.102	2.513	0.193	-0.087	0.048
P. fluorescens	9428	0.008	0.699	0.049	-0.011	0.005
P. fragi	NCIMB 8987	0.289	0.11	0.526	0.037	-0.022
P. maltophilia	10257	0.501	0.092	0.544	0.536	2.345
P. paucimobilis	11030	0.532	0.765	0.249	0.817	1.388
R. pickettii	11149	0.185	0.152	0.601	0.052	1.347
P. putida	10936	0.111	1.689	0.258	0.013	0.052
P. stutzeri	12262	0.009	0.296	0.28	-0.01	0.009
P. stutzeri	10475	-0.006	0.148	0.345	-0.015	-0.024
P. vesiculare	10900	0.032	2.535	0.306	1.047	2.727
S. spiritivorum	ATCC 33861	0.015	0.632	0.434	0.104	0.028
B. ambifaria	11351	0.057	0.27	0.499	0.077	0.042
B. andropogonis	1279	1.486	0.524	0.381	1.143	2.572
B. andropogonis	2126	0.421	0.925	0.277	0.079	0.337
B. caryphylli	2155	0.143	0.479	0.493	0.032	0.042
B. caryphylli	2156	0.302	0.077	0.085	0.006	0.019
B. dolosa	18941	0.297	0.128	0.229	-0.015	0.107
B. dolosa	18942	0.088	0.345	0.727	0.032	0.032
B. gladioli	11626	0.012	2.227	0.337	0.012	0.084
B. gladioli	18113	0.007	1.655	0.535	0.018	0.08
B.gladioli pv. alliicola	2121	0.064	2.781	0.16	0.017	0.05
B. gladioli pv. alliicola	6877	0.011	0.659	0.233	0.031	0.036
B.gladioli pv. gladioli	2216	0.006	1.035	0.235	0.008	0.034
B.gladioli pv. gladioli	6880	0.027	0.616	0.31	0.019	0.072
B. glumae	1277	0.05	1.084	0.186	0.045	0.043
B. glumae	2196	0.241	2.366	0.29	0	0.065
B. phenazinium	2247	1.175	2.524	0.385	0.053	0.11
B. phenazinium	6868	0.182	2.252	0.708	0.053	0.06
P. apista	16408	0.699	2.487	0.913	0.064	0.201

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference				
Strain	**Reference**	**AQ**	**AR**	**AS**	**AT**	**AU**
P. norimberensis	13019	0.668	2.854	1.541	0.137	0.194
P. norimberensis	16603	0.552	0.415	0.583	0.064	0.065
P. pnomenusa	18087	0.133	0.057	0.598	-0.008	0.023
P. pnomenusa	18817	0.151	0.007	0.232	-0.004	0.006
P. pulmonicola	18107	0.207	0.06	0.224	0.029	0.018
P. sputorum	18100	0.904	9.886	0.784	0.133	0.147
P. sputorum	18819	0.289	0.14	0.52	0.19	0.086
R. basilensis	18990	0.094	0.144	0.079	0.109	1.918
R. basilensis	19286	0.104	0.234	0.534	0.091	0.481
R. campinensis	19282	0.006	0.094	0.626	-0.004	0.026
R. campinensis	19283	0.871	1.313	0.396	0.134	0.589
R. eutropha	1190	0.929	0.876	0.988	0.041	0.598
R. eutropha	1194	0.042	0.175	0.181	0.112	2.174
R. gilardii	3399	0	0.05	0.382	0	0
R. gilardii	3400	-0.012	0.132	0.484	0.022	0.035
R. mannitolilytica	19090	1.137	9.862	2.399	0.428	0.577
R. metallidurans	1195	0.008	0.122	0.385	0.103	0.106
R. metallidurans	19290	-0.013	0.058	0.334	0.059	-0.004
R. paucula	3244	-0.001	0.22	0.314	0.061	0.012
R. paucula	3245	-0.042	0.643	0.123	0.048	0.077
R. pickettii	5942	0.07	0.082	0.363	0.052	-0.006
R. pickettii	6871	0.372	0.167	0.502	-0.007	0.727
R. solanacearum	2291	0.025	0.092	0.112	0.012	1.543
R. solanacearum	2293	0.006	0.081	0.708	-0.031	1.509
R. taiwanensis	19425	1.135	2.624	0.537	0.439	1.902
S. maltophilia	957	0.395	0.105	0.442	0.409	2.339
S. maltophilia	958	1.014	0.063	0.703	1.353	2.346
H_2O		0.01	0.011	-0.102	0.002	0.001
H_2O		0.01	0.014	-0.039	0.003	0.002
H_2O		0.012	0.005	-0.035	0.005	0.002
H_2O		0.01	0.007	0.018	0.004	0
H_2O		0.01	0.019	-0.022	0.005	-0.005
E. coli	10418	0.024	0.248	0.778	-0.006	-0.021
Enterobacter cloacae	11936	0.209	0.187	0.534	0.044	-0.011

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference		
Strain	**Reference**	**BN**	**BO**	**BR**
P. aeruginosa	2688	0.996	0.038	0.124
P. aeruginosa	2702	0.861	0.001	0.296
P. aeruginosa	2704	1.027	0.004	0.114
P. aeruginosa	2706	0.909	0.005	0.095
P. aeruginosa	2715	0.763	0	0.076
P. aeruginosa	2720	0.784	-0.003	0.052
P. aeruginosa	2737	1.105	0.008	0.095
P. aeruginosa	2739	0.866	0.012	0.006
P. aeruginosa	2741	0.923	-0.017	0.074
P. aeruginosa	2742	2.765	0.021	0.071
P. aeruginosa	2749	0.889	-0.005	0.13
P. aeruginosa	2772	1.015	0.007	0.177
P. aeruginosa	2775	0.634	0.013	0.068
P. aeruginosa	2776	0.683	-0.237	0.079
P. aeruginosa	2778	0.739	0.053	0.163
P. aeruginosa	2779	0.756	0.025	0.133
P. aeruginosa	2780	0.765	0.002	0.053
P. aeruginosa	2781	0.698	-0.019	0.058
P. aeruginosa	2782	0.749	-0.024	0.05
P. aeruginosa	2783	0.78	-0.01	0.083
P. aeruginosa	PS1	0.76	-0.009	0.084
P. aeruginosa	PS2	0.943	-0.019	0.144
P. aeruginosa	PS3	1.043	0.007	0.054
P. aeruginosa	PS4	0.981	-0.001	0.104
P. aeruginosa	PS5	0.68	0.016	0.129
P. aeruginosa	PS6	0.744	-0.001	0.001
P. aeruginosa	PS7	0.788	0.01	0.107
P. aeruginosa	PS8	0.797	-0.003	0.07
P. aeruginosa	PS9	0.772	-0.006	0.09
P. aeruginosa	PS10	0.735	-0.005	0.045
P. aeruginosa	PS11	0.816	-0.007	0.11
P. aeruginosa	PS12	0.862	-0.003	0.044
P. aeruginosa	PS13	0.856	0.011	0.013
P. aeruginosa	PS14	0.889	-0.013	0.057
P. aeruginosa	PS15	0.991	0.011	0.05
P. aeruginosa	PS16	0.99	0	0.1
P. aeruginosa	PS17	0.677	0.008	0.048
P. aeruginosa	PS18	0.718	0	0.053
P. aeruginosa	PS19	0.707	0.019	0.077
P. aeruginosa	PS20	0.775	0.019	0.106
P. aeruginosa	PS21	1.002	0.009	0.104
P. aeruginosa	PS22	0.782	0.004	0.089
P. aeruginosa	PS23	0.763	0.006	0.05
P. aeruginosa	PS24	0.777	0.009	0.04
P. aeruginosa	PS25	0.764	-0.007	0.081
P. aeruginosa	PS26	0.943	0.008	0.133
P. aeruginosa	PS27	0.979	0.004	0.053
P. aeruginosa	PS28	1.101	0.004	0.104
P. aeruginosa	PS29	0.797	0.017	0.058
P. aeruginosa	PS30	0.808	-0.007	0.067
P. aeruginosa	PS31	0.722	-0.002	0.094
P. aeruginosa	PS32	0.82	0.004	0.06

Appendix 3.3a (cont`d..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference		
Strain	**Reference**	**BN**	**BO**	**BR**
P. aeruginosa	PS33	0.87	0.011	0.137
P. aeruginosa	PS34	0.783	0.015	0.186
P. aeruginosa	PS35	0.749	0.017	0.118
P. aeruginosa	PS36	0.873	0	0.043
P. aeruginosa	PS37	0.858	0.012	0.056
P. aeruginosa	PS38	0.86	-0.003	0.057
P. aeruginosa	PS39	0.967	0.001	0.121
P. aeruginosa	PS40	1.335	-0.006	0.071
P. aeruginosa	PS41	0.691	0	0.018
P. aeruginosa	PS42	0.733	0.001	0.046
P. aeruginosa	PS43	0.733	-0.001	-0.003
P. aeruginosa	PS44	0.724	0.026	0.046
P. aeruginosa	PS45	2.442	0.02	0.107
P. aeruginosa	PS46	0.789	0.012	-0.219
P. aeruginosa	PS47	0.92	-0.003	0.078
P. aeruginosa	PS48	0.842	0.034	0.117
P. aeruginosa	PS49	0.851	0.005	0.047
P. aeruginosa	PS50	0.903	-0.189	0.067
P. aeruginosa	PS51	0.878	-0.002	0.098
P. aeruginosa	PS52	0.876	0	0.09
B. cepacia	LMG 1222	1.42	0.074	0.289
B. cepacia	LMG 2161	0.714	0.083	0.214
B. cenocepacia	LMG 16654	0.784	0.056	0.406
B. cenocepacia	LMG 16656	0.776	0.034	0.142
B. cenocepacia	LMG 16659	0.785	0.044	0.126
B. cepacia	LMG 17997	0.811	0.023	0.167
B. cepacia	LMG 18821	0.855	0.043	0.167
B. cenocepacia	LMG 18826	0.918	0.051	0.165
B. cenocepacia	LMG 18827	0.921	0.056	0.18
B. cenocepacia	LMG 18828	0.9	0.027	0.127
B. cenocepacia	LMG 18829	0.962	0.047	0.115
B. cenocepacia	LMG 18830	1.18	0.031	0.157
B. cenocepacia	LMG 18832	1.385	0.08	0.196
B. cenocepacia	LMG 18863	0.91	0.075	0.149
B. multivorans	LMG 13010	0.817	0.067	0.351
B. multivorans	LMG 16660	0.997	0.041	0.134
B. multivorans	LMG 16665	0.948	0.049	0.139
B. multivorans	LMG 17588	0.954	0.068	0.199
B. multivorans	LMG 18822	0.938	0.054	0.151
B. multivorans	LMG 18823	9.548	0.164	0.207
B. multivorans	LMG 18824	0.84	0.061	0.108
B. multivorans	LMG 18825	1.168	0.032	0.146
B. stabilis	LMG 14086	0.991	0.023	0.063
B. stabilis	LMG 14294	1.338	0.011	0.102
B. stabilis	LMG 18870	1.017	0.054	0.251
B. stabilis	LMG 18888	1.071	0.003	0.168
B. vietnamiensis	LMG 10929	1.183	0.073	0.275
B. vietnamiensis	LMG 16232	1.025	-0.037	0.204
B. vietnamiensis	LMG 18835	0.965	0.058	0.297
B. vietnamiensis	LMG 18836	1.127	0.013	0.188
A. baumannii	ATCC 19606	1.015	0.004	-0.003
A. calcoaceticus	7844	0.88	-0.004	0.049

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference		
Strain	**Reference**	**BN**	**BO**	**BR**
A. haemolyticus	12155	0.681	-0.009	-0.002
A. johnsonii	10308	1.054	0.004	0.062
A. lwoffi	5866	1.21	-0.002	-0.027
A. lwoffi	5867	2.019	0.022	0.087
A. lwoffi	NCIMB 12456	0.811	0.013	0.057
B diminuta	ATCC 11568	0.783	0.12	0.173
B. vesicularis	ATCC 11426	0.885	0.028	0.108
R. pickettii	11149	0.891	0.137	0.13
C. meningosepticum	ATCC 13253	1.109	0.9	0.221
M. nonliquefaciens	10464	0.989	-0.017	0.105
M. osloensis	10465	0.96	0.017	0.054
M. urethralis	11010	1.265	0.149	0.076
O. urethralis	11999	1.295	0.31	0.098
P. acidovorans	10683	1.04	-0.01	0.061
P. aeruginosa	6749	1.642	-0.02	0.014
P. aeruginosa	10332	0.9	0.005	0.138
P. alcaligenes	10367	0.591	-0.039	-0.046
P. pseudoalcaligenes	10860	0.721	0.025	0.075
P. diminuta	8545	1.062	0.63	0.135
P. fluorescens	10754	1.028	0.402	0.116
P. fluorescens	10392	0.925	0.013	0.045
P. fluorescens	3756	0.89	-0.003	0.144
P. fluorescens	10038	0.929	0.001	0.163
P. fluorescens	10688	0.892	0.033	0.205
P. fluorescens	9428	1.208	-0.008	0.004
P. fragi	NCIMB 8987	1.032	0.256	0.059
P. maltophilia	10257	0.965	0.192	0.116
P. paucimobilis	11030	0.821	0.018	0.026
R. pickettii	11149	0.964	0.017	0.277
P. putida	10936	0.757	0.027	0.019
P. stutzeri	12262	0.905	0.009	0.054
P. stutzeri	10475	0.803	0.009	0.019
P. vesiculare	10900	1.004	0.252	0.108
S. spiritivorum	ATCC 33861	1.039	0.34	0.094
B. ambifaria	11351	1.066	0.094	0.49
B. andropogonis	1279	0.949	0.165	0.186
B. andropogonis	2126	0.977	0.176	0.135
B. caryphylli	2155	1.475	0.077	0.066
B. caryphylli	2156	0.946	0.164	0.05
B. dolosa	18941	1.062	0.804	0.106
B. dolosa	18942	1.005	0.17	0.158
B. gladioli	11626	2.12	-0.001	0.209
B. gladioli	18113	0.964	0.023	0.267
B.gladioli pv. alliicola	2121	1.082	0.037	0.267
B. gladioli pv. alliicola	6877	0.985	0.037	0.264
B.gladioli pv. gladioli	2216	1.111	0.056	0.272
B.gladioli pv. gladioli	6880	1.159	0.062	0.242
B. glumae	1277	1.045	0.006	0.275
B. glumae	2196	1.478	0.036	0.229
B. phenazinium	2247	1.413	0.185	0.112
B. phenazinium	6868	0.867	0.253	0.064
P. apista	16408	0.998	0.162	0.054

Appendix 3.3a (cont'd..): Increases in absorbance (405 nm) caused by various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference		
Strain	**Reference**	**BN**	**BO**	**BR**
P. norimberensis	13019	1.116	0.784	0.105
P. norimberensis	16603	0.481	-0.002	-0.046
P. pnomenusa	18087	0.154	0.005	-0.06
P. pnomenusa	18817	0.471	-0.018	-0.037
P. pulmonicola	18107	0.355	-0.002	-0.018
P. sputorum	18100	1.167	0.424	0.101
P. sputorum	18819	0.621	0.038	-0.075
R. basilensis	18990	1.044	0.229	0.122
R. basilensis	19286	1.031	0.121	0.126
R. campinensis	19282	0.824	0.014	0.013
R. campinensis	19283	1.016	0.435	0.163
R. eutropha	1190	1.087	0.34	0.165
R. eutropha	1194	0.719	0.003	0.019
R. gilardii	3399	0.533	0.009	0.091
R. gilardii	3400	0.587	0.01	0.082
R. mannitolilytica	19090	1.364	1.297	0.069
R. metallidurans	1195	0.912	-0.002	0.197
R. metallidurans	19290	1.005	-0.004	0.152
R. paucula	3244	1.189	0.005	0.117
R. paucula	3245	1.157	0.024	0.116
R. pickettii	5942	1.2	0.016	0.308
R. pickettii	6871	1.304	0.006	0.211
R. solanacearum	2291	0.859	0.067	0.088
R. solanacearum	2293	0.891	0.006	0.092
R. taiwanensis	19425	1.283	0.91	0.074
S. maltophilia	957	1.087	0.233	0
S. maltophilia	958	0.998	0.284	0.035
H_2O		-0.069	0.004	-0.081
H_2O		-0.067	0.002	-0.092
H_2O		-0.083	0.002	-0.09
H_2O		-0.078	0.001	-0.081
H_2O		-0.054	0.002	-0.075
E. coli	10418	0.133	0.092	-0.016
Enterobacter cloacae	11936	0.1	0.121	-0.036

Appendix 3.3b: Key to chromogenic substrates

Substrate reference	Substrate
A	Ac-ala-ala-ala-*p* NA
B	H-glu-gly-arg-*pNA*
C	Suc-ala-ala-pro-phe-*pNA*
D	Suc-phe-leu-phe-*p* NA
E	H-ala-ala-phe-*p* NA
F	H-cys(bzl)-*p* NA
G	Ac-phe-*p* NA
H	H-gly-phe-*p* NA
I	Suc-ala-ala-ala-*p* NA
J	H-glu-gly-arg-*p* NA (duplicate)
K	Boc-leu-gly-arg-AMC
L	Ac-met-AMC
M	Ac-ala-ala-pro-ala-*p* NA
N	Suc-ala-leu-pro-phe-*p* NA
O	*p* NP-caprate
P	*p* NP-ß-arabinoside
Q	*p* NP-*p* -guanidinobenzoate
R	*p* NP-caprylate
S	*o* NP-myristate
T	*p* NP-α-rhamnoside
U	*p* NP-phosphorylcholine
V	*p* NP-phenyl phosphonate
W	*p* NP-α-fucoside
X	*p* NP-ß-lactoside
Y	BZ-DL-arg-*p* NA
Z	H-γ-glu-ala-gly-*p* NA
AB	*p* NP-α-maltoside
AC	*p* NP-ß-maltoside
AD	*p* NP-α-xyloside
AE	*p* NP-valerate
AQ	H-γ-glu-ala-gly-*p* NA (duplicate)
AR	H-ala-ala-phe-*p* NA (duplicate)
AS	H-cys(bzl)-*p* NA (duplicate)
AT	Suc-ala-ala-ala-*p* NA (duplicate)
AU	Suc-ala-leu-pro-phe-*p* NA (duplicate)
BN	*p* NP-caprate (duplicate)
BO	*p* NP-phenyl-phosphonate (duplicate)
BR	*p* NP-myristate

NB: For full substrate names see Materials section of Chapter 3.

Appendix 3.4: Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**M**	**N**	**O**
P. aeruginosa	2688	-	+/-	+	+/-	+/-	-	-	+/-	+/-	+	-	+	+++
P. aeruginosa	2702	-	++	+/-	+/-	-	-	-	-	+++	++	+/-	+	+++
P. aeruginosa	2704	-	+++	+/-	+/-	-	+/-	-	+/-	+++	++	-	+	+++
P. aeruginosa	2706	-	+/-	+	+	-	-	-	+/-	+	+	-	++	+++
P. aeruginosa	2715	-	+	-	-	++	+/-	-	+/-	-	+++	+	-	+++
P. aeruginosa	2720	-	-	-	-	+/-	-	-	+/-	-	+/-	-	-	+++
P. aeruginosa	2737	-	+++	-	-	++	+/-	-	+/-	-	+++	-	-	+++
P. aeruginosa	2739	-	++	-	-	-	-	-	+/-	-	+++	-	-	+++
P. aeruginosa	2741	-	-	-	-	+/-	-	-	+/-	-	-	-	-	+++
P. aeruginosa	2742	-	+	+/-	+/-	-	-	-	+/-	-	+	-	+/-	+++
P. aeruginosa	2749	-	+	-	-	++	+/-	-	+	-	+	-	-	+++
P. aeruginosa	2772	-	++	-	-	+	-	-	+/-	-	++	-	+/-	+++
P. aeruginosa	2775	-	++	-	-	-	+/-	-	+	-	+	-	-	+++
P. aeruginosa	2776	-	++	-	-	-	+/-	-	+/-	-	++	-	-	+++
P. aeruginosa	2778	-	-	-	+/-	+/-	-	-	+/-	-	++	-	-	+++
P. aeruginosa	2779	-	+/-	-	-	-	-	-	-	-	-	-	-	+++
P. aeruginosa	2780	-	+	-	-	-	-	-	-	-	+	-	-	+++
P. aeruginosa	2781	-	+/-	-	-	+	-	-	+/-	-	+/-	-	-	+++
P. aeruginosa	2782	-	+	-	-	+/-	+/-	-	+/-	-	+	-	-	+++
P. aeruginosa	2783	-	+	+/-	+/-	+/-	-	-	+/-	+/-	+	-	++	+++
P. aeruginosa	PS1	-	+	-	-	-	-	-	+/-	-	+/-	-	-	+++
P. aeruginosa	PS2	-	+	+/-	-	+	+/-	-	+/-	-	+/-	-	++	+++
P. aeruginosa	PS3	-	+/-	-	-	-	-	-	+/-	+++	++	-	++	+++
P. aeruginosa	PS4	-	+	-	+/-	-	-	-	+/-	+++	+	-	+/-	+++
P. aeruginosa	PS5	-	+	-	-	++	+/-	-	+/-	+/-	++	+/-	++	+++
P. aeruginosa	PS6	-	+	+	+/-	-	-	-	-	+++	++	+	+	+++
P. aeruginosa	PS7	-	+/-	+	-	+/-	-	-	+/-	+/-	+/-	+/-	+	+++
P. aeruginosa	PS8	-	-	-	-	-	-	-	-	++	+/-	-	-	+++
P. aeruginosa	PS9	-	+	+	+	+/-	-	-	+/-	+++	+++	-	++	+++
P. aeruginosa	PS10	-	+	-	+/-	-	-	-	-	++	++	+	+	+++
P. aeruginosa	PS11	-	+/-	-	-	++	-	-	+/-	-	-	-	-	+++
P. aeruginosa	PS12	-	+	+/-	+/-	-	-	-	-	+++	+	-	+	+++
P. aeruginosa	PS13	-	++	+/-	-	-	-	-	-	+	++	-	++	+++
P. aeruginosa	PS14	-	+	-	-	++	-	-	-	-	++	-	-	+++
P. aeruginosa	PS15	-	+	+/-	-	-	-	-	-	+	++	-	++	+++
P. aeruginosa	PS16	-	+	+	+	-	-	-	-	+	+	-	++	+++
P. aeruginosa	PS17	-	-	+/-	-	-	-	-	+/-	-	+	++	+	+++
P. aeruginosa	PS18	-	+	+	+	+/-	-	-	+/-	+++	+	++	++	+++
P. aeruginosa	PS19	-	+	+	+	+	+/-	-	+/-	++	+	++	++	+++
P. aeruginosa	PS20	-	+	-	-	-	+/-	-	-	-	++	+++	+	+++
P. aeruginosa	PS21	-	+	-	-	-	+/-	-	-	+/-	+	++	+	+++
P. aeruginosa	PS22	-	+/-	+/-	-	-	-	-	-	+++	++	+	++	+++
P. aeruginosa	PS23	-	++	+/-	-	-	+/-	-	-	++	++	-	+	+++
P. aeruginosa	PS24	-	+	-	-	+	+/-	-	-	-	+	-	-	+++
P. aeruginosa	PS25	-	-	-	-	+	-	-	-	-	-	-	-	+++
P. aeruginosa	PS26	-	-	+	+/-	-	-	-	+/-	+++	++	+	+++	+++
P. aeruginosa	PS27	-	-	+	+/-	+/-	-	-	+/-	-	+/-	-	+/-	+++
P. aeruginosa	PS28	-	-	+	+/-	-	-	-	-	+++	++	-	+/-	+++

Appendix 3.4 (cont`d..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**M**	**N**	**O**
P. aeruginosa	PS29	-	++	+	-	+/-	-	-	+/-	+	+	++	+	+++
P. aeruginosa	PS30	-	-	-	-	++	-	-	+/-	-	+	++	-	+++
P. aeruginosa	PS31	-	+	+/-	+/-	-	-	-	+/-	++	+	++	+	+++
P. aeruginosa	PS32	-	-	+/-	-	-	-	-	-	++	++	++	++	+++
P. aeruginosa	PS33	-	-	-	-	++	-	-	+/-	-	-	+	-	+++
P. aeruginosa	PS34	-	++	+/-	-	-	+/-	-	+/-	+/-	++	++	+	+++
P. aeruginosa	PS35	-	-	-	-	+/-	-	-	+/-	-	-	+	+	+++
P. aeruginosa	PS36	-	+	+/-	+/-	-	-	-	+/-	+++	++	+	+	+++
P. aeruginosa	PS37	-	+	-	-	++	-	-	+/-	-	++	++	-	+++
P. aeruginosa	PS38	-	+	+/-	+	-	-	-	+/-	+++	++	+	+	+++
P. aeruginosa	PS39	-	+	+/-	+	-	-	-	+/-	+/-	++	++	+	+++
P. aeruginosa	PS40	-	-	+/-	+/-	-	-	-	+/-	+	+	-	+	+++
P. aeruginosa	PS41	-	+	-	-	-	-	-	+/-	-	+/-	++	-	+++
P. aeruginosa	PS42	-	+	+/-	-	-	-	-	+/-	++	++	++	+	+++
P. aeruginosa	PS43	-	+/-	+	+/-	-	-	-	+/-	-	++	++	-	+++
P. aeruginosa	PS44	-	+	-	-	-	+/-	-	+/-	-	++	+++	-	+++
P. aeruginosa	PS45	-	+	-	-	+	-	-	+/-	-	++	++	-	+++
P. aeruginosa	PS46	-	+	-	-	-	-	-	+/-	-	+	++	-	+++
P. aeruginosa	PS47	-	+	-	-	+	-	-	+/-	+/-	++	++	+	+++
P. aeruginosa	PS48	-	-	-	-	++	-	-	+/-	-	-	+	-	+++
P. aeruginosa	PS49	-	++	+	+	-	-	-	+/-	+++	+++	+	+	+++
P. aeruginosa	PS50	-	+	-	-	++	-	-	+/-	-	++	++	-	+++
P. aeruginosa	PS51	-	-	+	+	-	-	-	+/-	+/-	+/-	+/-	++	+++
P. aeruginosa	PS52	-	-	-	-	+++	-	-	+/-	-	-	+	-	+++
B. cepacia	LMG 1222	-	-	-	+	+/-	-	-	+	-	-	+/-	-	+++
B. cepacia	LMG 2161	-	-	-	-	-	-	-	+/-	-	-	+	-	+++
B. cenocepacia	LMG 16654	-	-	-	+/-	+/-	-	-	+/-	-	-	+/-	-	+++
B. cenocepacia	LMG 16656	-	-	-	+/-	+/-	-	-	+/-	-	-	+/-	-	+++
B. cenocepacia	LMG 16659	-	-	-	+/-	+/-	-	-	+/-	+/-	-	+/-	-	+++
B. cepacia	LMG 17997	-	-	-	+	+/-	+/-	-	+	-	-	+/-	-	+++
B. cepacia	LMG 18821	-	-	-	-	-	-	-	-	-	+/-	+/-	+/-	+++
B. cenocepacia	LMG 18826	-	+	-	-	+/-	-	-	+/-	-	+/-	+/-	+/-	+++
B. cenocepacia	LMG 18827	-	+/-	-	-	+/-	-	-	+/-	+	+	++	+++	+++
B. cenocepacia	LMG 18828	-	+	-	+	+/-	-	-	+/-	-	+	++	-	+++
B. cenocepacia	LMG 18829	-	-	-	+	+	+/-	-	+	-	-	-	-	+++
B. cenocepacia	LMG 18830	-	-	-	+/-	-	+/-	-	-	-	+	++	+	+++
B. cenocepacia	LMG 18832	-	++	-	+/-	-	+/-	-	+/-	-	-	+/-	-	+++
B. cenocepacia	LMG 18863	-	-	-	-	-	-	-	-	-	-	+/-	-	+++
B. multivorans	LMG 13010	-	+	++	+	+/-	-	-	+/-	+++	+	+/-	++	+++
B. multivorans	LMG 16660	-	++	++	+	+/-	-	-	+/-	++	+	++	++	+++
B. multivorans	LMG 16665	-	-	-	+	+/-	+/-	-	+	-	-	++	-	+++
B. multivorans	LMG 17588	-	-	-	-	+++	+/-	-	++	-	-	-	-	+++
B. multivorans	LMG 18822	-	-	-	-	+++	+/-	-	+++	-	-	+/-	-	+++
B. multivorans	LMG 18823	-	+	-	+/-	+++	++	-	++	-	+	+	-	+++
B. multivorans	LMG 18824	-	-	-	-	-	-	-	+/-	-	-	-	-	+++
B. multivorans	LMG 18825	-	-	-	-	-	-	-	+/-	-	+/-	++	-	+++
B. stabilis	LMG 14086	-	+/-	-	-	-	-	-	-	-	-	-	-	+++
B. stabilis	LMG 14294	-	-	-	-	+++	+/-	-	+++	-	+	+++	-	+++

Appendix 3.4 (cont`d..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**M**	**N**	**O**
B. stabilis	LMG 18870	-	-	+/-	+/-	+/-	+/-	-	+/-	+/-	-	-	-	+++
B. stabilis	LMG 18888	-	-	-	-	+/-	-	-	-	-	-	-	-	+++
B. vietnamiensis	LMG 10929	-	-	-	-	+/-	+	-	+/-	-	+/-	+	-	+++
B. vietnamiensis	LMG 16232	-	-	-	-	-	-	-	-	-	-	-	-	+++
B. vietnamiensis	LMG 18835	-	-	-	-	-	+	-	+/-	-	-	-	-	+++
B. vietnamiensis	LMG 18836	-	-	-	-	-	-	-	+/-	-	-	+/-	-	+++
A. baumannii	ATCC 19606	-	-	-	-	+/-	-	-	+/-	-	-	-	-	+++
A. calcoaceticus	7844	-	-	-	-	+	-	-	+/-	-	-	-	-	+++
A. haemolyticus	12155	-	+/-	+/-	-	+/-	-	-	+/-	-	+	-	-	+++
A. johnsonii	10308	-	-	-	-	+	-	-	+	-	-	+/-	-	+++
A. lwoffi	5866	-	-	-	-	+	-	-	+/-	-	-	-	-	+++
A. lwoffi	5867	-	+	-	-	+/-	-	-	+/-	-	-	-	-	+++
A. lwoffi	NCIMB 12456	-	-	-	-	-	-	-	-	-	-	-	-	+++
B diminuta	ATCC 11568	-	+++	+++	+++	++	+/-	-	+++	+	++	++	+++	+++
B. vesicularis	ATCC 11426	-	+++	+++	+++	+++	-	-	+++	+	++	++	+++	+++
R. pickettii	11149	-	-	++	-	-	-	-	-	-	-	-	+++	+++
C. meningosepticum	ATCC 13253	-	+++	+++	+	+++	+++	-	+++	+	+++	++	++	+++
M. nonliquefaciens	10464	-	+/-	-	-	+++	-	-	-	-	-	-	+++	+++
M. osloensis	10465	-	+	+/-	+/-	+++	+	-	++	+	+++	++	+	+++
M. urethralis	11010	-	-	-	-	-	-	-	-	-	-	-	-	+++
O. urethralis	11999	-	++	+	+/-	+++	++	-	+++	+	+++	++	+	+++
P. acidovorans	10683	-	-	-	-	-	-	-	-	-	-	-	-	+++
P. aeruginosa	6749	-	-	-	-	-	-	-	-	-	+/-	+	-	+++
P. aeruginosa	10332	-	+/-	++	+/-	+	-	-	+/-	-	-	-	++	+++
P. alcaligenes	10367	-	+/-	+++	-	-	-	-	-	+/-	+	++	+++	+++
P. pseudoalcaligenes	10860	-	-	-	-	+	+/-	-	+	-	-	-	-	+++
P. diminuta	8545	-	+++	+++	+++	+++	-	-	+++	+	+++	++	+++	+++
P. fluorescens	10754	-	+++	+++	+	+++	++	-	+++	+/-	++	+	+	+++
P. fluorescens	10392	-	+++	+++	-	-	-	-	-	-	++	+	++	+++
P. fluorescens	3756	-	++	-	-	+/-	-	-	+	-	+	+/-	-	+++
P. fluorescens	10038	-	+	-	-	-	-	-	-	-	-	-	-	+++
P. fluorescens	10688	-	++	-	-	+++	-	-	+++	-	++	+	-	+++
P. fluorescens	9428	-	-	+++	+/-	+++	-	-	+++	-	-	+	+++	+++
P. fragi	NCIMB 8987	-	-	-	-	+/-	-	-	-	-	-	-	-	+/-
P. maltophilia	10257	-	+++	+++	-	-	-	-	+++	+/-	+++	++	+++	+++
P. paucimobilis	11030	-	++	+++	+	+	+	+/-	+	++	+++	+++	+++	+++
R. pickettii	11149	-	-	+	-	+/-	-	-	-	-	-	-	+++	+++
P. putida	10936	-	+	+++	-	+++	+/-	-	+++	-	-	-	-	+++
P. stutzeri	12262	-	+	+/-	-	++	+/-	-	++	-	+/-	-	-	+++
P. stutzeri	10475	-	+	+++	-	+	+/-	-	+	-	+++	++	+++	+++
P. vesiculare	10900	-	+++	+++	+++	++	-	-	+++	+	+++	+++	+++	+++
S. spiritivorum	ATCC 33861	-	++	-	-	+	+/-	-	+/-	-	+	+/-	-	+++
B. ambifaria	11351	-	-	-	+	-	-	-	+/-	-	-	-	-	+++
B. andropogonis	1279	-	+++	++	-	-	-	-	+++	+/-	++	++	++	+++
B. andropogonis	2126	-	++	+	+/-	+++	+	-	+++	+/-	++	+	++	+++
B. caryphylli	2155	-	-	+/-	-	++	-	-	++	-	+	+	+	+++
B. caryphylli	2156	-	-	-	-	+/-	+	-	+	-	-	-	-	-
B. dolosa	18941	-	-	-	-	+/-	-	-	-	-	-	-	-	-

Appendix 3.4 (cont`d..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**M**	**N**	**O**
B. dolosa	18942	-	+	+/-	-	+++	+	-	+++	-	-	-	-	-
B. gladioli	11626	-	+	+/-	-	+/-	+	-	+++	-	-	-	-	-
B. gladioli	18113	-	-	-	-	-	-	-	+++	-	-	-	-	+++
B.gladioli pv. alliicola	2121	-	+/-	-	-	+/-	-	-	+++	-	-	+/-	+++	+++
B. gladioli pv. alliicola	6877	-	-	-	-	+/-	-	-	+++	-	-	-	-	+++
B.gladioli pv. gladioli	2216	-	-	-	-	+/-	-	-	+++	-	-	-	++	+++
B.gladioli pv. gladioli	6880	-	+/-	-	-	-	-	-	+++	-	-	-	-	+++
B. glumae	1277	-	-	-	-	+/-	-	-	+++	-	-	-	-	+++
B. glumae	2196	-	-	-	-	+/-	-	-	+++	-	-	-	-	+++
B. phenazinium	2247	-	+	+/-	-	+++	+	-	+++	+/-	++	++	+	+++
B. phenazinium	6868	-	+++	++	+/-	+++	++	-	+++	+	+++	++	+	+++
P. apista	16408	-	-	-	-	-	-	-	-	-	-	-	-	+++
P. norimberensis	13019	-	+++	++	+/-	+++	+++	-	+++	-	+/-	+/-	-	+++
P. norimberensis	16603	-	-	-	-	-	-	-	-	-	-	+/-	-	+++
P. pnomenusa	18087	-	+/-	-	-	-	-	-	-	-	-	-	-	+/-
P. pnomenusa	18817	-	+/-	+	-	-	-	-	-	-	-	-	-	+/-
P. pulmonicola	18107	-	-	-	-	-	-	-	-	-	-	-	-	+++
P. sputorum	18100	-	-	-	-	-	-	-	-	-	-	-	-	+++
P. sputorum	18819	-	-	-	-	-	-	-	-	-	+/-	+/-	-	+++
R. basilensis	18990	-	-	-	-	-	-	-	-	-	-	-	-	+++
R. basilensis	19286	-	-	-	-	-	-	-	-	-	-	-	-	+++
R. campinensis	19282	-	-	-	-	-	-	-	-	-	-	-	-	+++
R. campinensis	19283	-	-	-	-	-	-	-	-	-	-	+/-	-	+++
R. eutropha	1190	-	++	+++	+/-	++	+	-	+	+/-	++	++	+++	+++
R. eutropha	1194	-	-	-	-	-	-	-	-	-	-	-	-	+++
R. gilardii	3399	-	-	-	-	-	+	-	+/-	-	-	-	-	+++
R. gilardii	3400	-	+/-	-	-	-	-	-	-	-	-	-	-	+++
R. mannitolilytica	19090	-	++	++	+/-	+++	+++	-	+++	+/-	++	+/-	+/-	+++
R. metallidurans	1195	-	+/-	-	-	-	-	-	-	-	-	-	-	+++
R. metallidurans	19290	-	-	-	-	-	-	-	-	-	-	+/-	-	+++
R. paucula	3244	-	+	+/-	-	+	+/-	-	+	-	-	-	-	+++
R. paucula	3245	-	-	-	-	-	-	-	-	-	+/-	+/-	-	+++
R. pickettii	5942	-	-	-	-	-	-	-	-	-	-	-	-	+++
R. pickettii	6871	-	+	+/-	-	+/-	-	-	+/-	-	-	-	+++	+++
R. solanacearum	2291	-	+	-	-	+	+/-	-	+	-	-	++	-	+++
R. solanacearum	2293	-	+++	+/-	+/-	++	+	-	++	-	-	+	-	+++
R. taiwanensis	19425	-	+++	++	+	+++	++	-	+++	+/-	++	+/-	+	+++
S. maltophilia	957	-	+++	+++	+/-	-	-	-	++	++	+++	+++	+++	+++
S. maltophilia	958	-	+++	+++	-	-	-	-	++	+	+++	++	+++	+++
H_2O		-	-	-	-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	10418	-	+/-	-	-	-	-	-	+/-	-	-	-	-	-
Enterobacter cloacae	11936	-	-	-	-	-	-	-	+/-	-	+/-	-	-	-

Appendix 3.4 (cont'd..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**AE**	**AD**	**AB**	**AC**
P. aeruginosa	2688	-	+	+++	++	-	+	-	-	-	+++	-	-	-
P. aeruginosa	2702	-	+	+++	++	-	++	-	-	-	+++	-	-	-
P. aeruginosa	2704	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	2706	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	2715	-	+	+++	++	-	++	-	-	-	+++	-	-	-
P. aeruginosa	2720	-	+	+++	+	-	+/-	-	-	-	+++	-	-	-
P. aeruginosa	2737	-	+	+++	++	-	+	-	-	-	+++	-	-	-
P. aeruginosa	2739	-	+	+++	+	-	++	-	-	-	+++	-	-	-
P. aeruginosa	2741	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	2742	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	2749	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	2772	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	2775	-	+	+++	+	-	+/-	-	-	-	+++	-	-	-
P. aeruginosa	2776	-	+	+++	++	-	+	-	-	-	+++	-	-	-
P. aeruginosa	2778	-	+	+++	++	-	++	-	+/-	-	+++	-	-	-
P. aeruginosa	2779	-	+	+++	+	-	++	-	+	-	+++	-	-	-
P. aeruginosa	2780	-	+	+++	+	-	+	-	-	-	+++	-	-	-
P. aeruginosa	2781	-	+	+++	+	-	+	-	-	-	+++	-	-	-
P. aeruginosa	2782	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	2783	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS1	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS2	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS3	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS4	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS5	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS6	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS7	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS8	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS9	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS10	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS11	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS12	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS13	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS14	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS15	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS16	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS17	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS18	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS19	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS20	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS21	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS22	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS23	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS24	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS25	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS26	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	PS27	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS28	-	+	+++	++	-	+++	-	-	-	+++	-	-	-

Appendix 3.4 (cont'd..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**AE**	**AD**	**AB**	**AC**
P. aeruginosa	PS29	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS30	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS31	-	+	+++	++	-	++	-	-	-	+++	-	-	-
P. aeruginosa	PS32	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS33	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS34	-	+/-	+++	++	-	+++	-	+	-	+++	-	-	-
P. aeruginosa	PS35	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	PS36	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS37	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS38	-	+/-	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS39	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS40	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS41	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS42	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS43	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS44	-	+	+++	+	-	+/-	-	-	-	+++	-	-	-
P. aeruginosa	PS45	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS46	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS47	-	+	+++	+	-	+	-	-	-	+++	-	-	-
P. aeruginosa	PS48	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	PS49	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	PS50	-	+	+++	++	-	-	-	-	-	+++	-	-	-
P. aeruginosa	PS51	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
P. aeruginosa	PS52	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cepacia	LMG 1222	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cepacia	LMG 2161	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 16654	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 16656	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 16659	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cepacia	LMG 17997	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cepacia	LMG 18821	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 18826	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 18827	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 18828	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 18829	-	+	+++	+++	-	+++	+/-	-	-	+++	-	-	-
B. cenocepacia	LMG 18830	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 18832	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. cenocepacia	LMG 18863	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. multivorans	LMG 13010	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. multivorans	LMG 16660	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
B. multivorans	LMG 16665	-	+/-	+++	++	-	+++	-	-	-	+++	-	-	-
B. multivorans	LMG 17588	-	+/-	+++	++	-	-	-	-	-	+++	-	-	-
B. multivorans	LMG 18822	-	+/-	+++	++	-	-	-	-	-	+++	-	-	-
B. multivorans	LMG 18823	-	+/-	+++	++	-	-	-	-	-	+++	-	-	-
B. multivorans	LMG 18824	-	+	+++	++	-	-	-	-	-	+++	-	-	-
B. multivorans	LMG 18825	-	+	+++	++	-	-	-	-	-	+++	-	-	-
B. stabilis	LMG 14086	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. stabilis	LMG 14294	-	+	+++	++	-	-	-	-	-	+++	-	-	-

Appendix 3.4 (cont`d..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**AE**	**AD**	**AB**	**AC**
B. stabilis	LMG 18870	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. stabilis	LMG 18888	-	+	+++	++	-	+++	-	-	+/-	+++	-	-	-
B. vietnamiensis	LMG 10929	-	+	+++	++	-	-	-	-	-	+++	-	-	-
B. vietnamiensis	LMG 16232	-	+	+++	++	-	+	+/-	-	-	+++	-	-	-
B. vietnamiensis	LMG 18835	-	+	+++	+	-	-	-	-	-	+++	-	-	-
B. vietnamiensis	LMG 18836	-	+	+++	++	-	+/-	-	-	-	+++	-	-	-
A. baumannii	ATCC 19606	-	+	+++	-	-	-	-	-	-	+++	+/-	-	-
A. calcoaceticus	7844	-	+	+++	-	-	-	-	-	-	+++	+/-	-	-
A. haemolyticus	12155	-	+	+++	+	-	+	-	-	-	+++	+/-	-	-
A. johnsonii	10308	-	+	+++	-	-	-	-	-	-	+++	-	-	-
A. lwoffi	5866	-	+	+++	+/-	-	-	-	-	-	+++	+/-	-	-
A. lwoffi	5867	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
A. lwoffi	NCIMB 12456	-	+	+++	+	-	++	-	-	-	+++	-	-	+/-
B diminuta	ATCC 11568	-	+	+++	+	-	-	+/-	-	-	+++	-	-	-
B. vesicularis	ATCC 11426	-	+	+++	-	-	-	-	-	-	+++	-	+	-
R. pickettii	11149	-	+++	+++	++	-	-	-	-	-	+++	-	+	-
C. meningosepticum	ATCC 13253	-	+	+++	+/-	-	+/-	+	++	+	+++	-	++	+
M. nonliquefaciens	10464	-	+	+++	++	-	++	-	+/-	-	+++	-	-	-
M. osloensis	10465	-	+	+++	+/-	-	-	-	+/-	-	+++	-	+/-	-
M. urethralis	11010	-	+	+++	+	-	+	-	+	-	+	-	-	-
O. urethralis	11999	-	+	+++	+	-	+	+/-	+	-	+++	-	++	-
P. acidovorans	10683	-	+	+++	+/-	-	-	-	+/-	-	+++	-	-	-
P. aeruginosa	6749	-	+	+++	+	-	-	-	-	-	+++	-	+/-	-
P. aeruginosa	10332	-	+	+++	++	-	+	-	-	-	+++	-	-	-
P. alcaligenes	10367	-	+	+++	+	-	+	-	-	-	+++	-	++	+
P. pseudoalcaligenes	10860	-	+	+++	+/-	-	-	-	-	-	+++	-	-	-
P. diminuta	8545	-	+	+++	-	-	+	+/-	-	-	+++	-	+	-
P. fluorescens	10754	-	+	+++	+	-	++	+/-	++	-	+++	-	++	-
P. fluorescens	10392	-	+	+++	-	-	-	-	-	-	+++	-	+	-
P. fluorescens	3756	-	+	+++	-	-	-	-	+/-	-	+++	-	+	-
P. fluorescens	10038	-	+	+++	-	-	-	-	-	-	+++	-	-	-
P. fluorescens	10688	-	+	+++	-	-	+	-	-	-	+++	-	-	-
P. fluorescens	9428	-	+	+++	+/-	-	-	-	-	-	+++	-	-	-
P. fragi	NCIMB 8987	-	+	-	-	-	-	+/-	-	-	++	-	-	-
P. maltophilia	10257	-	+	+++	+	-	-	+/-	-	-	+++	-	+++	+/-
P. paucimobilis	11030	-	+	+++	+	-	+	-	+/-	++	+++	+/-	+/-	+/-
R. pickettii	11149	-	+++	+++	++	-	-	-	-	-	+++	-	+	-
P. putida	10936	-	+	++	-	-	-	-	-	-	+++	-	-	-
P. stutzeri	12262	-	+	+++	+	-	-	-	+	-	+++	-	-	-
P. stutzeri	10475	-	+	+++	+	-	++	-	+	-	+++	-	+++	-
P. vesiculare	10900	-	+	+++	+	-	-	-	-	-	+++	-	+	-
S. spiritivorum	ATCC 33861	-	+	+++	+/-	-	-	-	+	+	+++	+/-	++	+++
B. ambifaria	11351	-	+	+++	++	-	+	-	-	-	+++	-	-	+/-
B. andropogonis	1279	-	+	+++	++	-	-	+	++	-	+++	-	+++	+/-
B. andropogonis	2126	-	+	+++	-	-	-	+/-	+	+/-	+++	+/-	++	+++
B. caryphylli	2155	-	+	+++	++	-	+++	-	+	-	+++	-	-	-
B. caryphylli	2156	-	+	+++	++	-	-	-	-	-	+++	-	+/-	+/-
B. dolosa	18941	-	+	+++	+/-	-	-	+	-	+/-	+++	-	+	++

Appendix 3.4 (cont`d..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference												
Strain	**Reference**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**AE**	**AD**	**AB**	**AC**
B. dolosa	18942	-	+	+++	++	-	+/-	+	+	+/-	+++	-	++	+
B. gladioli	11626	-	+	+++	++	-	+++	-	-	-	+++	-	++	-
B. gladioli	18113	-	+	+++	++	-	+++	-	+	-	+++	-	-	-
B.gladioli pv. alliicola	2121	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. gladioli pv. alliicola	6877	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B.gladioli pv. gladioli	2216	-	+	+++	+	-	+++	-	-	-	+++	-	-	-
B.gladioli pv. gladioli	6880	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
B. glumae	1277	-	+	+++	++	-	-	-	-	-	+++	-	-	-
B. glumae	2196	-	+	+++	++	-	-	-	-	-	+++	-	-	-
B. phenazinium	2247	-	+	+++	-	-	+/-	-	-	-	+++	-	+++	-
B. phenazinium	6868	-	+	+++	+	-	+	-	-	-	+++	-	-	-
P. apista	16408	-	+	+++	-	-	-	-	-	-	+++	-	-	-
P. norimberensis	13019	-	+	+++	-	-	-	-	-	-	+++	-	+++	+/-
P. norimberensis	16603	-	+	+++	-	-	-	-	-	-	+++	-	-	+/-
P. pnomenusa	18087	-	+	+	-	-	-	++	-	-	+++	-	-	-
P. pnomenusa	18817	-	+	+	-	-	-	-	-	-	+++	-	-	-
P. pulmonicola	18107	-	+	+	+/-	-	-	-	-	-	+++	-	-	-
P. sputorum	18100	-	+	+++	-	-	-	-	+/-	-	+++	-	-	-
P. sputorum	18819	-	+	+++	-	-	-	-	-	-	+++	-	-	-
R. basilensis	18990	-	+	+++	+	-	-	-	-	-	+++	-	-	-
R. basilensis	19286	-	+	+++	-	-	-	-	-	-	+++	-	-	-
R. campinensis	19282	-	++	+++	-	-	-	-	-	-	+++	-	-	-
R. campinensis	19283	-	++	+++	-	-	-	-	-	-	+++	-	-	-
R. eutropha	1190	-		+++	+	-	-	+/-	+	+	+++	-	++	++
R. eutropha	1194	-		+++	++	-	-	-	-	-	+++	-	-	-
R. gilardii	3399	-		+++	-	-	-	-	-	-	+++	-	-	-
R. gilardii	3400	-		+++	-	-	-	-	-	-	+++	-	-	-
R. mannitolilytica	19090	-	++	+++	+	-	+/-	+/-	++	-	+++	-	++	+/-
R. metallidurans	1195	-	++	+++	+	-	-	-	-	-	+++	-	-	-
R. metallidurans	19290	-	++	+++	+	-	-	-	-	-	+++	-	-	-
R. paucula	3244	-	+	+++	++	-	-	-	-	-	+++	-	-	-
R. paucula	3245	-	+	+++	+	-	-	-	-	-	+++	-	-	-
R. pickettii	5942	-	+++	+++	++	-	-	-	-	-	+++	-	-	-
R. pickettii	6871	-	+	+++	++	-	-	-	-	-	+++	-	-	-
R. solanacearum	2291	-	+	+++	++	-	+++	-	-	-	+++	-	-	-
R. solanacearum	2293	-	+	+++	-	-	-	-	-	-	+++	-	+++	-
R. taiwanensis	19425	-	+	+++	++	-	+++	+	++	-	+++	-	++	-
S. maltophilia	957	-	+	+++	-	-	-	-	-	-	+++	-	+++	+
S. maltophilia	958	-	+	+++	+	-	-	+	-	-	+++	-	+++	+/-
H_2O		-	+	-	-	-	-	-	-	-	+	-	-	-
H_2O		-	+	-	-	-	-	-	-	-	+	-	-	-
H_2O		-	+	-	-	-	-	-	-	-	+	-	-	-
H_2O		-	+	-	-	-	-	-	-	-	+	-	-	-
H_2O		-	+	-	-	-	-	-	-	-	+	-	-	-
E. coli	10418	-	+	++	-	-	-	+/-	-	-	+	-	-	-
Enterobacter cloacae	11936	-	+	++	-	-	-	+/-	-	+	+	-	-	-

Appendix 3.4 (cont'd..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference									
Strain	**Reference**	**Y**	**Z**	**AQ**	**AR**	**AS**	**AT**	**AU**	**BN**	**BO**	**BR**
P. aeruginosa	2688	+/-	+/-	+/-	+++	-	-	+/-	++	-	+
P. aeruginosa	2702	+/-	+/-	+/-	-	-	+	+/-	++	-	+
P. aeruginosa	2704	-	-	-	-	-	-	+/-	++	-	+
P. aeruginosa	2706	+/-	+/-	+	+/-	-	-	+/-	++	-	+
P. aeruginosa	2715	+/-	+/-	+/-	+	-	-	-	++	-	+
P. aeruginosa	2720	+/-	-	-	-	-	-	-	++	-	+
P. aeruginosa	2737	+/-	-	-	+/-	-	-	-	++	-	+
P. aeruginosa	2739	+/-	-	-	-	-	-	-	++	-	+
P. aeruginosa	2741	+/-	-	-	-	-	-	-	++	-	+
P. aeruginosa	2742	-	+/-	+/-	+	-	-	-	++	-	+
P. aeruginosa	2749	+/-	-	+/-	+	-	-	-	++	-	+
P. aeruginosa	2772	-	+/-	+/-	+/-	-	-	-	++	-	+
P. aeruginosa	2775	+/-	+/-	-	-	-	-	-	++	-	+
P. aeruginosa	2776	+/-	+/-	+/-	+/-	-	-	-	++	-	+
P. aeruginosa	2778	+/-	-	-	+/-	-	-	-	++	-	+
P. aeruginosa	2779	+/-	+	+/-	-	-	-	-	++	-	+
P. aeruginosa	2780	-	-	-	+	-	-	-	++	-	+
P. aeruginosa	2781	+/-	+/-	-	+/-	-	-	-	++	-	+
P. aeruginosa	2782	+/-	+/-	-	-	-	-	-	++	-	+
P. aeruginosa	2783	-	-	+/-	++	-	-	-	++	-	+
P. aeruginosa	PS1	-	-	+/-	+++	-	-	-	++	-	+
P. aeruginosa	PS2	-	-	-	++	-	-	-	++	-	+
P. aeruginosa	PS3	-	-	+/-	-	-	+	+/-	++	-	+
P. aeruginosa	PS4	-	+	+/-	-	-	+	+/-	++	-	+
P. aeruginosa	PS5	+/-	+	++	+++	-	-	-	++	-	+
P. aeruginosa	PS6	-	-	-	-	-	+	+/-	++	-	+
P. aeruginosa	PS7	+/-	+/-	-	-	-	-	-	++	-	+
P. aeruginosa	PS8	-	+/-	-	-	-	+	-	++	-	+
P. aeruginosa	PS9	-	+/-	+	-	-	+	+/-	++	-	+
P. aeruginosa	PS10	-	-	-	-	-	-	-	++	-	+
P. aeruginosa	PS11	+/-	+	+	+++	-	-	-	++	-	+
P. aeruginosa	PS12	-	-	-	-	-	+	+/-	++	-	+
P. aeruginosa	PS13	-	-	-	+/-	-	-	-	++	-	+
P. aeruginosa	PS14	-	-	+/-	+/-	-	-	-	++	-	+
P. aeruginosa	PS15	-	-	+/-	++	-	-	-	++	-	+
P. aeruginosa	PS16	-	+/-	+/-	++	-	-	-	++	-	+
P. aeruginosa	PS17	+/-	+/-	+	++	-	-	+++	++	-	+
P. aeruginosa	PS18	+/-	+/-	-	-	-	+/-	-	++	-	+
P. aeruginosa	PS19	+/-	+/-	+/-	++	-	-	++	++	-	+
P. aeruginosa	PS20	+/-	+/-	+/-	+/-	-	-	++	++	-	+
P. aeruginosa	PS21	-	-	-	-	-	-	++	++	-	+
P. aeruginosa	PS22	+/-	+/-	-	-	-	+/-	-	++	-	+
P. aeruginosa	PS23	-	-	-	+/-	-	-	++	++	-	+
P. aeruginosa	PS24	+/-	-	-	+/-	-	-	-	++	-	+
P. aeruginosa	PS25	+/-	+	+	++	+/-	-	+++	++	-	+
P. aeruginosa	PS26	-	+	-	+	-	-	-	++	-	+
P. aeruginosa	PS27	-	+/-	+/-	-	-	+/-	+/-	++	-	+
P. aeruginosa	PS28	-	+/-	-	-	-	+/-	-	++	-	+

Appendix 3.4 (cont'd..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference									
Strain	**Reference**	**Y**	**Z**	**AQ**	**AR**	**AS**	**AT**	**AU**	**BN**	**BO**	**BR**
P. aeruginosa	PS29	+/-	-	-	+	-	-	++	++	-	+
P. aeruginosa	PS30	+/-	+/-	+/-	++	-	-	++	++	-	+
P. aeruginosa	PS31	+/-	-	-	+	-	-	+	++	-	+
P. aeruginosa	PS32	+/-	-	-	-	-	-	++	++	-	+
P. aeruginosa	PS33	+/-	+/-	+/-	+++	-	-	++	++	-	+
P. aeruginosa	PS34	+/-	-	-	+	-	-	+++	++	-	+
P. aeruginosa	PS35	+/-	-	+/-	+/-	-	-	+++	++	-	+
P. aeruginosa	PS36	-	-	-	-	-	+/-	-	++	-	+
P. aeruginosa	PS37	-	-	+	++	-	-	+++	++	-	+
P. aeruginosa	PS38	-	-	-	-	-	-	-	++	-	+
P. aeruginosa	PS39	-	-	+/-	++	-	-	-	++	-	+
P. aeruginosa	PS40	-	-	+/-	+	-	+/-	+/-	++	-	+
P. aeruginosa	PS41	+/-	+/-	+	-	-	-	+++	++	-	-
P. aeruginosa	PS42	+/-	+/-	-	+/-	-	-	+/-	++	-	+
P. aeruginosa	PS43	+/-	+/-	+/-	+	-	-	+/-	++	-	+
P. aeruginosa	PS44	+/-	-	-	-	-	-	+++	++	-	+
P. aeruginosa	PS45	+/-	+/-	+	++	-	-	+++	+++	-	+
P. aeruginosa	PS46	-	-	-	+/-	-	-	+/-	++	-	+
P. aeruginosa	PS47	+/-	-	-	+/-	-	-	+/-	++	-	+
P. aeruginosa	PS48	-	+/-	+/-	-	-	-	+/-	++	-	+
P. aeruginosa	PS49	-	-	-	+/-	-	+/-	-	++	-	+
P. aeruginosa	PS50	-	+/-	+/-	++	-	-	+++	++	-	+
P. aeruginosa	PS51	-	+/-	-	+	-	-	+/-	++	-	+
P. aeruginosa	PS52	-	+/-	+/-	++	-	-	+++	++	-	+
B. cepacia	LMG 1222	+/-	+/-	-	-	-	-	-	+++	-	+
B. cepacia	LMG 2161	-	-	-	-	-	-	-	++	-	+
B. cenocepacia	LMG 16654	+/-	+/-	-	-	-	-	-	++	-	+
B. cenocepacia	LMG 16656	-	+/-	-	-	-	-	-	++	-	+
B. cenocepacia	LMG 16659	-	-	-	-	-	-	-	++	-	+
B. cepacia	LMG 17997	-	+/-	-	-	-	-	+/-	++	-	+
B. cepacia	LMG 18821	-	-	-	-	-	-	++	++	-	+
B. cenocepacia	LMG 18826	-	-	-	-	-	-	+/-	++	-	+
B. cenocepacia	LMG 18827	-	-	-	+/-	-	-	+/-	++	-	+
B. cenocepacia	LMG 18828	-	+/-	-	-	-	-	-	++	-	+
B. cenocepacia	LMG 18829	-	+/-	-	-	-	-	+/-	++	-	+
B. cenocepacia	LMG 18830	-	-	-	-	-	-	-	++	-	+
B. cenocepacia	LMG 18832	-	+/-	++	+	+/-	-	+++	+++	-	+
B. cenocepacia	LMG 18863	-	+/-	-	-	-	-	+++	++	-	+
B. multivorans	LMG 13010	-	+/-	-	-	-	-	-	++	-	+
B. multivorans	LMG 16660	-	-	-	-	-	-	-	++	-	+
B. multivorans	LMG 16665	-	+/-	-	-	-	-	-	++	-	+
B. multivorans	LMG 17588	-	-	-	+/-	-	-	+/-	++	-	+
B. multivorans	LMG 18822	-	-	-	+/-	-	-	+/-	++	-	+
B. multivorans	LMG 18823	-	-	-	-	-	-	-	+++	+/-	+
B. multivorans	LMG 18824	-	-	-	-	-	-	+/-	++	-	+
B. multivorans	LMG 18825	-	-	-	-	-	-	++	++	-	+
B. stabilis	LMG 14086	-	-	-	-	-	-	-	++	-	+
B. stabilis	LMG 14294	-	-	-	-	-	-	+/-	++	-	+

Appendix 3.4 (cont'd..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference									
Strain	**Reference**	**Y**	**Z**	**AQ**	**AR**	**AS**	**AT**	**AU**	**BN**	**BO**	**BR**
B. stabilis	LMG 18870	-	-	-	-	-	-	-	++	-	+
B. stabilis	LMG 18888	-	+/-	-	-	-	-	-	++	-	+
B. vietnamiensis	LMG 10929	-	+++	-	-	-	-	-	++	-	+
B. vietnamiensis	LMG 16232	-	-	-	+/-	-	-	-	++	+/-	+
B. vietnamiensis	LMG 18835	-	-	-	-	-	-	-	++	+/-	+
B. vietnamiensis	LMG 18836	-	-	-	-	-	-	-	++	-	+
A. baumannii	ATCC 19606	-	-	-	-	-	-	-	++	-	-
A. calcoaceticus	7844	-	-	-	-	-	-	-	++	-	-
A. haemolyticus	12155	-	-	-	-	-	-	-	++	-	+/-
A. johnsonii	10308	-	-	-	-	-	-	-	++	-	+/-
A. lwoffi	5866	-	-	-	-	-	-	-	++	-	-
A. lwoffi	5867	-	-	-	+/-	-	-	-	++	-	+
A. lwoffi	NCIMB 12456	-	-	-	-	-	-	+/-	++	-	+/-
B diminuta	ATCC 11568	+++	-	-	+	-	+/-	+	++	+/-	+
B. vesicularis	ATCC 11426	+++	-	-	+++	-	+	+++	++	-	+/-
R. pickettii	11149	-	-	-	-	-	+	+++	++	-	+/-
C. meningosepticum	ATCC 13253	++	+	+	+++	++	+	+/-	++	+	+/-
M. nonliquefaciens	10464	-	+	+	+++	-	-	-	++	-	+
M. osloensis	10465	-	+/-	-	+++	+/-	-	+/-	++	-	+/-
M. urethralis	11010	-	-	-	-	-	-	-	++	+/-	+/-
O. urethralis	11999	+	++	+++	+++	+	-	-	++	+/-	+
P. acidovorans	10683	-	+	+/-	-	-	-	-	++	-	+/-
P. aeruginosa	6749	-	+/-	+/-	-	-	-	-	++	-	-
P. aeruginosa	10332	-	+/-	+	+++	-	-	-	++	-	+
P. alcaligenes	10367	-	-	-	+++	-	-	-	++	-	-
P. pseudoalcaligenes	10860	-	-	-	+/-	-	-	-	++	-	+
P. diminuta	8545	+++	-	-	+++	-	++	+++	++	+	+
P. fluorescens	10754	+	++	++	+++	+	+	++	++	+	+
P. fluorescens	10392	+	+/-	-	-	-	-	-	++	-	-
P. fluorescens	3756	+	+/-	-	+++	-	-	-	++	-	+
P. fluorescens	10038	-	-	+/-	-	-	-	-	++	-	+
P. fluorescens	10688	-	-	-	+++	-	-	-	++	-	-
P. fluorescens	9428	+/-	-	-	+/-	-	-	-	++	-	+
P. fragi	NCIMB 8987	-	+	+/-	-	-	-	-	+/-	+	+
P. maltophilia	10257	+	+	+	-	-	++	+++	++	+/-	+/-
P. paucimobilis	11030	+/-	-	+	+	+	++	+++	++	-	+/-
R. pickettii	11149	-	+/-	-	-	-	-	+++	++	-	+
P. putida	10936	-	-	-	++	-	-	-	++	-	-
P. stutzeri	12262	+/-	-	-	-	-	-	-	++	-	+
P. stutzeri	10475	+/-	-	-	-	-	-	-	++	-	+/-
P. vesiculare	10900	++	+/-	-	+++	-	++	+++	++	+	+
S. spiritivorum	ATCC 33861	++	+/-	-	+/-	-	-	-	++	+	+
B. ambifaria	11351	-	-	-	-	-	-	-	++	-	+
B. andropogonis	1279	++	++	+++	+/-	-	++	+++	++	+	+
B. andropogonis	2126	++	++	+/-	+	+/-	-	-	++	+	-
B. caryphylli	2155	-	-	-	-	+/-	-	-	++	+/-	+
B. caryphylli	2156	+	+	+/-	-	-	-	-	++	+/-	+/-
B. dolosa	18941	+/-	+	+/-	-	-	-	-	++	+	+/-

Appendix 3.4 (cont`d..): Colour intensity recordings of various bacteria due to hydrolysis of chromogenic substrates after 48 hr incubation.

		Substrate reference									
Strain	**Reference**	**Y**	**Z**	**AQ**	**AR**	**AS**	**AT**	**AU**	**BN**	**BO**	**BR**
B. dolosa	18942	+/-	+	-	-	-	-	-	++	+	+
B. gladioli	11626	-	-	-	+++	-	-	-	++	-	+
B. gladioli	18113	-	-	-	+++	-	-	-	++	-	+
B.gladioli pv. alliicola	2121	-	-	-	+++	-	-	-	++	-	+
B. gladioli pv. alliicola	6877	-	-	-	+/-	-	-	-	++	-	+
B.gladioli pv. gladioli	2216	-	-	-	+/-	-	-	-	++	-	+
B.gladioli pv. gladioli	6880	-	-	-	+/-	-	-	-	++	-	+
B. glumae	1277	-	-	-	+	-	-	-	++	-	+
B. glumae	2196	-	-	-	+++	-	-	-	++	-	+
B. phenazinium	2247	+/-	+++	+++	+++	+/-	-	-	++	+/-	+/-
B. phenazinium	6868	-	-	+/-	+++	+/-	-	-	++	+/-	+/-
P. apista	16408	-	-	+	+++	+/-	-	-	++	+/-	+/-
P. norimberensis	13019	++	-	+	+++	++	+/-	-	++	+	+/-
P. norimberensis	16603	-	+/-	+	-	+/-	-	-	+/-	-	-
P. pnomenusa	18087	-	+/-	-	-	-	-	-	+/-	-	-
P. pnomenusa	18817	-	-	-	-	-	-	-	+/-	-	-
P. pulmonicola	18107	-	+/-	-	-	-	-	-	+/-	-	-
P. sputorum	18100	-	-	+	+++	+	+/-	-	++	+	+
P. sputorum	18819	-	-	-	-	-	-	-	++	-	-
R. basilensis	18990	-	-	-	-	-	-	+++	++	+/-	+/-
R. basilensis	19286	-	-	-	-	-	-	+/-	++	-	+/-
R. campinensis	19282	-	-	-	-	-	-	-	++	-	-
R. campinensis	19283	-	-	++	++	+/-	+/-	+	++	+	+
R. eutropha	1190	+	+	++	++	+/-	-	+	++	+	+
R. eutropha	1194	-	-	-	-	+/-	-	+++	++	-	+/-
R. gilardii	3399	-	-	-	-	-	-	-	++	-	+/-
R. gilardii	3400	-	-	-	-	-	-	-	++	-	-
R. mannitolilytica	19090	++	++	++	+++	+++	+	+/-	++	++	+/-
R. metallidurans	1195	-	-	-	-	-	-	-	++	-	+/-
R. metallidurans	19290	-	-	-	-	-	-	-	++	-	+/-
R. paucula	3244	-	-	-	-	-	-	-	++	-	+
R. paucula	3245	-	-	-	+/-	-	-	-	++	-	+
R. pickettii	5942	-	-	-	-	-	-	-	++	-	+
R. pickettii	6871	-	-	+/-	-	-	-	+	++	-	+
R. solanacearum	2291	-	-	-	-	-	-	+++	++	-	-
R. solanacearum	2293	-	-	-	-	-	-	+++	++	-	-
R. taiwanensis	19425	+	+	++	+++	+	+	+++	++	+	+/-
S. maltophilia	957	++	+	+/-	-	-	+	+++	++	+/-	-
S. maltophilia	958	+	+	++	-	-	++	+++	++	+/-	+/-
H_2O		-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-
H_2O		-	-	-	-	-	-	-	-	-	-
E. coli	10418	+/-	-	-	+/-	-	-	-	+/-	+/-	-
Enterobacter cloacae	11936	-	+/-	+/-	+/-	-	-	-	+/-	+/-	-

NB: For key to chromogenic substrates see Appendix 3.3b

Appendix 3.5a: Cumulative percentages of organisms hydrolysing glycosidase enzyme substrates which showed no differentiation between organisms

Organism	No. of strains	Glycosidase substrate					
		MU-α-ara	MU-ß-gal	MU-α-glu	MU-ß-gur	MU-α-man	MU-ß-man
Acinetobacter sp.	7	0	0	0	0	0	0
Brevindumonas sp.	2	0	0	50	0	0	0
Bcc	33	0	3	3	0	0	0
Bcc species							
B. cepacia	4	0	25	25	0	0	0
B. multivorans	8	0	0	0	0	0	0
B. cenocepacia	10	0	0	0	0	0	0
B. stabilis	4	0	0	0	0	0	0
B. vietnamiensis	4	0	0	0	0	0	0
B. dolosa	2	0	0	0	0	0	0
B. ambifaria	1	0	0	0	0	0	0
Burkholderia gladioli	6	0	0	17	0	0	0
other *Burkholderia* sp.	9	11	22	11	0	0	0
C. meningosepticum	1	0	100	100	0	0	100
Moraxella sp.	3	0	0	67	0	0	0
Oligella sp.	1	0	0	100	0	0	0
Pandoraea sp.	8	0	0	0	0	0	0
P. aeruginosa	74	0	0	1	0	0	1
other *Pseudomonas* sp.	17	12	12	41	0	6	0
Ralstonia pickettii	4	0	0	0	0	0	0
other *Ralstonia* sp.	16	0	0	6	6	0	0
S. spiritivorum	1	100	100	100	0	100	100
S. maltophilia	2	0	0	50	0	100	0

Key: MU-α-arab,4-methylumbelliferyl-α-L-arabinoside; MU-ß-gal, 4-methylumbelliferyl-ß-D-galactoside; MU-α-glu, 4-methylumbelliferyl-α-D-glucoside; MU-ß-gur, 4-methylumbelliferyl-ß-D-glucuronide; MU-α-man,4-methylumbelliferyl-α-D-manoside; MU-ß-man, 4-methylumbelliferyl-ß-D-mannoside

Appendix 3.5b: Cumulative percentages of organisms hydrolysing aminopeptidase enzyme substrates which showed no differentiation between organisms

Organism	No. of strains	Aminopeptidase substrate				
		L-ala-AMC	L-arg-AMC	Z-arg-AMC	L-asp-AMC	L-asn-AMC
Acinetobacter sp.	7	100	100	0	0	71
Brevindumonas sp.	2	100	100	0	0	50
Bcc	33	100	97	0	0	58
Bcc species						
B. cepacia	4	100	100	0	0	25
B. multivorans	8	100	100	0	0	88
B. cenocepacia	10	100	100	0	0	70
B. stabilis	4	100	100	0	0	50
B. vietnamiensis	4	100	100	0	0	25
B. dolosa	2	100	100	0	0	50
B. ambifaria	1	100	100	0	0	0
Burkholderia gladioli	6	100	100	0	0	67
other *Burkholderia* sp.	9	89	89	11	11	56
C. meningosepticum	1	100	100	0	100	100
Moraxella sp.	3	100	100	0	0	67
Oligella sp.	1	100	100	0	0	100
Pandoraea sp.	8	100	63	0	0	38
P. aeruginosa	74	99	99	0	0	9
other *Pseudomonas* sp.	17	100	94	0	6	29
Ralstonia pickettii	4	100	100	0	0	75
other *Ralstonia* sp.	16	100	94	0	0	44
S. spiritivorum	1	100	100	0	100	100
S. maltophilia	2	100	100	0	0	0

Key: L-ala-AMC , L-alanyl-7-amido-4-methylcoumarin; L-arg-AMC, L-arginyl-7-amido-4-methylcoumarin
Z-arg-AMC, Z-arginyl-7-amido-4-methylcoumarin; L-asp-AMC, L-aspartyl-7-amido-4-methylcoumarin
L-asn-AMC, L-asparaginyl-7-amido-4-methylcoumarin

Appendix 3.5b (cont`d..): Cumulative percentages of organisms hydrolysing aminopeptidase enzyme substrates which showed no differentiation between organisms

Organism	No. of strains	Aminopeptidase substrate			
		L-gln-AMC	L-glu-AMC	gly-AMC	L-ile-AMC
Acinetobacter sp.	7	100	100	100	86
Brevindumonas sp.	2	100	100	100	50
Bcc	33	88	100	100	6
Bcc species					
B. cepacia	4	75	100	100	0
B. multivorans	8	100	100	100	13
B. cenocepacia	10	70	100	100	0
B. stabilis	4	100	100	100	0
B. vietnamiensis	4	100	100	100	25
B. dolosa	2	100	100	100	0
B. ambifaria	1	100	100	100	0
Burkholderia gladioli	6	100	100	83	50
other *Burkholderia* sp.	9	89	89	89	56
C. meningosepticum	1	100	100	100	100
Moraxella sp.	3	100	100	100	0
Oligella sp.	1	100	100	100	0
Pandoraea sp.	8	100	100	100	13
P. aeruginosa	74	99	95	100	1
other *Pseudomonas* sp.	17	88	53	88	29
Ralstonia pickettii	4	100	100	100	0
other *Ralstonia* sp.	16	100	94	94	0
S. spiritivorum	1	100	100	100	100
S. maltophilia	2	100	100	100	50

Key: L-gln-AMC, L-glutamyl-7-amido-4-methylcoumarin; L-glu-AMC, L-glutamic acid-7-amido-4-methylcoumarin; gly-AMC, glycyl-7-amido-4-methylcoumarin; L-ile-AMC, L-isoleucyl-7-amido-4-methylcoumarin

Appendix 3.5b (cont`d..): Cumulative percentages of organisms hydrolysing aminopeptidase substrates which showed no differentiation between organisms

Organism	No. of strains	Aminopeptidase substrate				
		L-leu-AMC	L-lys-AMC	L-phe-AMC	L-pro-AMC	L-tyr-AMC
Acinetobacter sp.	7	100	100	100	43	86
Brevindumonas sp.	2	100	100	100	100	100
Bcc	33	88	100	97	100	100
Bcc species						
B. cepacia	4	50	100	100	100	100
B. multivorans	8	100	100	100	100	100
B. cenocepacia	10	80	100	90	100	100
B. stabilis	4	100	100	100	100	100
B. vietnamiensis	4	100	100	100	100	100
B. dolosa	2	100	100	100	100	100
B. ambifaria	1	100	100	100	100	100
Burkholderia gladioli	6	100	100	100	100	100
other *Burkholderia* sp.	9	89	89	89	89	89
C. meningosepticum	1	100	100	100	100	100
Moraxella sp.	3	100	100	100	100	100
Oligella sp.	1	100	100	100	100	100
Pandoraea sp.	8	100	50	38	88	100
P. aeruginosa	74	99	99	96	100	100
other *Pseudomonas* sp.	17	100	100	88	82	88
Ralstonia pickettii	4	100	100	100	100	100
other *Ralstonia* sp.	16	100	88	100	100	88
S. spiritivorum	1	100	100	100	0	100
S. maltophilia	2	100	100	50	50	0

Key: L-leu-AMC, L-leucyl-7-amido-4-methylcoumarin; L-lys-AMC, L-lysyl-7-amido-4-methylcoumarin; L-phe-AMC, L-phenylalanyl-7-amido-4-methylcoumarin; L-pro-AMC, L-prolyl-7-amido-4-methylcoumarin; L-tyr-AMC, L-tyrosyl-7-amido-4-methylcoumarin

Appendix 3.5c: Cumulative percentages of organisms hydrolysing one esterase and α- and β- trypsin substrates which showed no differentiation between organisms

Organism	No. of strains	Esterase substrate							
		MU-ace	MU-prop	MU-but	MU-hep	MU-non	MU-lau	MU-ela	MU-guano
Acinetobacter sp.	**7**	100	100	100	100	100	100	86	71
Brevindumonas sp.	**2**	100	100	100	100	100	100	50	50
Bcc	**33**	100	100	100	100	100	100	100	0
Bcc species									
B. cepacia	**4**	100	100	100	100	100	100	100	0
B. multivorans	**8**	100	100	100	100	100	100	100	0
B. cenocepacia	**10**	100	100	100	100	100	100	100	0
B. stabilis	**4**	100	100	100	100	100	100	100	0
B. vietnamiensis	**4**	100	100	100	100	100	100	100	0
B. dolosa	**2**	100	100	100	100	100	100	100	0
B. ambifaria	**1**	100	100	100	100	100	100	100	0
Burkholderia gladioli	**6**	100	100	100	100	100	100	100	0
other *Burkholderia* sp.	**9**	89	89	89	89	89	89	67	0
C. meningosepticum	**1**	100	100	100	100	100	100	0	0
Moraxella sp.	**3**	100	100	100	67	67	100	33	0
Oligella sp.	**1**	100	100	100	0	0	100	100	0
Pandoraea sp.	**8**	100	100	100	13	13	25	0	0
P. aeruginosa	**74**	100	100	100	100	100	100	99	0
other *Pseudomonas* sp.	**17**	100	100	100	100	94	94	59	0
Ralstonia pickettii	**4**	100	100	100	100	100	100	100	25
other *Ralstonia* sp.	**16**	100	100	100	100	88	100	38	0
S. spiritivorum	**1**	100	100	100	100	100	100	0	100
S. maltophilia	**2**	100	100	100	100	100	100	50	0

Key: MU-ace, 4-methylumbelliferyl acetate; MU-prop,4-methylumbelliferyl propionate;
MU-but, 4-methylumbelliferyl butyrate; MU-hep, 4-methylumbelliferyl heptanoate;
MU-non, 4-methylumbelliferyl nonanoate; MU-lau, 4-methylumbelliferyl laurate;
MU-ela, 4-methylumbelliferyl elaidate; MU-guano, 4-methylumbelliferyl-p-guanidinobenzoate

Appendix 3.5d: Cumulative percentages of organisms hydrolysing endopeptidase enzyme substrates which showed no differentiation between organisms

Organism	No of strains	Endopeptidase substrate				
		Suc-ala-ala-pro-phe-*p* NA	H-gly-phe-*p* NA	Boc-leu-gly-arg-AMC	Ac-ala-ala-pro-ala-*p* NA	Bz-DL-arg-*p* Na
Acinetobacter sp.	7	0	14	86	0	0
Brevindumonas sp.	2	100	100	100	100	100
Bcc	33	6	27	42	30	0
Bcc species						
B. cepacia	4	0	50	25	25	0
B. multivorans	8	25	50	63	50	0
B. cenocepacia	10	0	10	30	30	0
B. stabilis	4	0	25	50	25	0
B. vietnamiensis	4	0	0	50	25	0
B. dolosa	2	0	50	0	0	0
B. ambifaria	1	0	0	100	0	0
Burkholderia gladioli	6	0	100	17	0	0
other *Burkholderia* sp.	8	38	100	63	63	38
C. meningosepticum	1	100	100	100	100	100
Moraxella sp	3	0	0	67	33	0
Oligella sp.	1	100	100	100	100	100
Pandoraea sp.	8	25	13	25	0	13
P. aeruginosa	74	22	3	72	45	0
other *Pseudomonas* sp.	17	59	71	88	59	35
Ralstonia picketti	4	50	0	100	0	0
other *Ralstonia* sp.	16	19	38	63	19	19
S. spiritivorum	1	0	0	100	0	100
S. maltophilia	2	100	100	100	100	100

Appendix 3.5d (cont`d..): Cumulative percentages of organisms hydrolysing miscellaneous chromogenic enzyme substrates which showed no differentiation between organisms

Organism	No of strains	miscellaneous chromogenic substrates			
		*p*NP caprylate	*p*NP α-fucoside	*p*NP ß-lactoside	*p*NP valerate
Acinetobacter sp.	7	100	0	100	100
Brevindumonas sp.	2	100	0	100	100
Bcc	33	100	3	100	100
Bcc species					
B. cepacia	4	100	0	0	100
B. multivorans	8	100	0	0	100
B. cenocepacia	10	100	0	0	100
B. stabilis	4	100	0	0	100
B. vietnamiensis	4	100	0	0	100
B. dolosa	2	100	50	0	100
B. ambifaria	1	100	0	0	100
Burkholderia gladioli	6	100	17	100	100
other *Burkholderia* sp.	8	100	38	100	100
C. meningosepticum	1	100	100	100	100
Moraxella sp	3	100	33	100	100
Oligella sp.	1	100	100	100	100
Pandoraea sp.	8	100	0	100	100
P. aeruginosa	74	100	3	100	100
other *Pseudomonas* sp.	17	94	18	94	100
Ralstonia picketti	4	100	0	100	100
other *Ralstonia* sp.	16	100	19	94	100
S. spiritivorum	1	100	100	100	100
S. maltophilia	2	100	0	100	100

NB: For full substrate names see Materials section of Chapter 3.

Appendix 3.6a: Colour intensity recordings for Carboxypeptidase substrates

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
P. aeruginosa	2688	-	-	-	-	-	-	-
P. aeruginosa	2702	-	-	-	-	-	-	-
P. aeruginosa	2704	-	-	-	-	-	+/-	+
P. aeruginosa	2706	-	-	-	-	-	-	-
P. aeruginosa	2715	-	-	-	-	-	-	-
P. aeruginosa	2720	-	-	-	-	-	-	-
P. aeruginosa	2737	+	-	-	-	-	+/-	-
P. aeruginosa	2739	+/-	-	-	-	-	-	-
P. aeruginosa	2741	+	-	-	-	-	-	-
P. aeruginosa	2742	+/-	-	-	-	-	-	-
P. aeruginosa	2749	++	-	-	-	-	-	-
P. aeruginosa	2772	-	-	-	-	-	-	-
P. aeruginosa	2775	+	-	-	-	-	-	-
P. aeruginosa	2776	+/-	+/-	-	-	-	-	-
P. aeruginosa	2778	++	+/-	-	-	-	-	-
P. aeruginosa	2779	+	-	-	-	-	-	-
P. aeruginosa	2780	-	-	-	-	-	-	-
P. aeruginosa	2781	-	-	+	-	-	-	-
P. aeruginosa	2782	-	-	-	-	-	-	-
P. aeruginosa	2783	-	-	-	-	-	-	-
P. aeruginosa	PS1	-	-	++	-	-	-	-
P. aeruginosa	PS2	-	-	-	-	-	-	-
P. aeruginosa	PS3	-	-	-	-	-	-	-
P. aeruginosa	PS4	-	-	-	-	-	-	-
P. aeruginosa	PS5	-	-	-	-	-	-	-
P. aeruginosa	PS6	-	-	-	-	-	-	-
P. aeruginosa	PS7	-	-	-	-	-	-	-
P. aeruginosa	PS8	-	-	-	-	-	-	-
P. aeruginosa	PS9	-	-	-	-	-	-	-
P. aeruginosa	PS10	-	-	-	-	-	-	-
P. aeruginosa	PS11	+++	+	-	-	-	+	-
P. aeruginosa	PS12	-	-	-	-	-	-	-
P. aeruginosa	PS13	-	-	-	-	-	-	-
P. aeruginosa	PS14	-	-	-	-	-	-	-
P. aeruginosa	PS15	-	-	-	-	-	-	-
P. aeruginosa	PS16	-	-	-	-	-	-	-
P. aeruginosa	PS17	-	-	-	-	-	-	-
P. aeruginosa	PS18	-	-	-	-	-	-	-
P. aeruginosa	PS19	-	-	-	-	-	-	-
P. aeruginosa	PS20	-	-	-	-	-	-	-
P. aeruginosa	PS21	-	-	-	-	-	-	-
P. aeruginosa	PS22	-	-	-	-	-	-	-
P. aeruginosa	PS23	-	-	-	-	-	-	-
P. aeruginosa	PS24	-	-	-	-	-	-	-
P. aeruginosa	PS25	-	-	-	-	-	-	-
P. aeruginosa	PS26	-	-	-	-	-	-	-
P. aeruginosa	PS27	-	-	-	-	-	-	-
P. aeruginosa	PS28	-	-	-	-	-	-	-
P. aeruginosa	PS29	-	-	-	-	-	-	-
P. aeruginosa	PS30	+/-	-	-	-	-	-	-
P. aeruginosa	PS31	-	-	-	-	-	-	-

Appendix 3.6a (cont`d..): Colour intensity recordings for Carboxypeptidase substrates

Strain	Reference	Substrate						
		ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
P. aeruginosa	PS32	-	-	-	-	-	-	-
P. aeruginosa	PS33	-	-	-	-	-	-	-
P. aeruginosa	PS34	+/-	-	-	-	-	-	-
P. aeruginosa	PS35	+/-	-	-	-	-	-	-
P. aeruginosa	PS36	-	-	-	-	-	-	-
P. aeruginosa	PS37	-	-	-	-	-	-	-
P. aeruginosa	PS38	-	-	-	-	-	-	-
P. aeruginosa	PS39	-	-	-	-	-	-	-
P. aeruginosa	PS40	-	-	-	-	-	-	-
P. aeruginosa	PS41	-	-	-	-	-	-	-
P. aeruginosa	PS42	-	-	-	-	-	-	-
P. aeruginosa	PS43	-	-	-	-	-	-	-
P. aeruginosa	PS44	-	-	-	-	-	-	-
P. aeruginosa	PS45	-	-	-	-	-	-	-
P. aeruginosa	PS46	-	-	-	-	-	-	-
P. aeruginosa	PS47	-	-	-	-	-	-	-
P. aeruginosa	PS48	-	-	-	-	-	-	-
P. aeruginosa	PS49	-	-	-	-	-	-	-
P. aeruginosa	PS50	-	-	-	-	-	-	-
P. aeruginosa	PS51	-	-	-	-	+	-	-
P. aeruginosa	PS52	-	-	-	-	-	-	-
B. cepacia	LMG 1222	+++	++	-	+++	-	++	+/-
B. cepacia	LMG 2161	+++	++	-	+++	-	++	+/-
B. cenocepacia	LMG 16654	+++	++	-	+/-	-	+	+
B. cenocepacia	LMG 16656	+++	++	-	+/-	-	+	+/-
B. cenocepacia	LMG 16659	+++	+/-	-	+++	-	+/-	-
B. cepacia	LMG 17997	+++	+/-	-	+	-	+/-	+/-
B. cepacia	LMG 18821	-	-	-	-	-	-	-
B. cenocepacia	LMG 18826	+++	+++	-	+	-	+	+
B. cenocepacia	LMG 18827	-	-	-	-	-	-	-
B. cenocepacia	LMG 18828	+++	+/-	-	-	-	+/-	-
B. cenocepacia	LMG 18829	+++	+/-	-	-	-	+	-
B. cenocepacia	LMG 18830	+/-	-	-	-	-	-	-
B. cenocepacia	LMG 18832	+	-	-	-	-	+	-
B. cenocepacia	LMG 18863	+++	+	-	+	-	+	-
B. multivorans	LMG 13010	+++	+/-	+/-	+/-	-	+	-
B. multivorans	LMG 16660	+++	++	-	+	-	++	+
B. multivorans	LMG 16665	+++	+	-	+/-	-	+	+/-
B. multivorans	LMG 17588	+++	+	-	-	-	+	-
B. multivorans	LMG 18822	+++	+++	-	-	+/-	++	-
B. multivorans	LMG 18823	+++	+/-	-	-	-	-	-
B. multivorans	LMG 18824	+++	+	-	+/-	-	+	-
B. multivorans	LMG 18825	+++	+	-	-	-	+/-	-
B. stabilis	LMG 14086	+++	+	-	-	-	++9	-
B. stabilis	LMG 14294	-	-	-	-	-	-	-
B. stabilis	LMG 18870	+++	+	-	-	-	+	+/-
B. stabilis	LMG 18888	+++	++	-	+++	+/-	+++	-

Appendix 3.6a (cont`d..): Colour intensity recordings for Carboxypeptidase substrates

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
B. vietnamiensis	LMG 10929	+++	+/-	-	+++	-	+/-	-
B. vietnamiensis	LMG 16232	+	+/-	-	-	-	+/-	-
B. vietnamiensis	LMG 18835	+	+++	-	-	-	+	-
B. vietnamiensis	LMG 18836	+++	+	-	+	-	+/-	-
A. baumannii	ATCC 19606	+++	+++	+++	+++	-	+++	+++
A. calcoaceticus	7844	+++	+++	+	+++	-	+++	+++
A. haemolyticus	12155	+++	+++	-	+/-	-	++	+++
A. johnsonii	10308	+/-	-	-	-	-	-	-
B. vesicularis	ATCC 11426	-	-	+/-	-	++	+	++
R. pickettii	11149	+++	+++	+++	+	+++	+++	+++
C. meningosepticum	ATCC 13253	-	-	-	-	-	-	-
M. nonliquefaciens	10464	-	-	-	-	-	-	-
M. osloensis	10465	+++	+/-	-	+/-	-	++	++
M. urethralis	11010	-	-	-	-	-	-	-
O. urethralis	11999	-	-	-	-	-	-	-
P. acidovorans	10683	+++	+++	+++	++	-	++	+++
P. aeruginosa	6749	-	-	-	-	-	-	-
P. aeruginosa	10332	-	-	-	-	-	-	-
P. alcaligenes	10367	+++	-	-	-	-	-	-
P. pseudoalcaligenes	10860	-	-	-	-	-	-	-
P. diminuta	8545	+++	+++	+++	-	+++	++	++
P. fluorescens	10754	++	+	-	-	+/-	+	-
P. fluorescens	10392	++	-	-	-	-	-	-
P. fluorescens	3756	+	-	-	-	-	-	-
P. fluorescens	10038	-	-	+	-	-	-	-
P. fluorescens	10688	-	-	-	-	-	+/-	-
P. fluorescens	9428	-	-	+++	-	-	-	-
P. fragi	NCIMB 8987	+	+++	-	-	-	-	-
P. maltophilia	10257	++	++	+	-	+/-	+	-
P. paucimobilis	11030	+	++	-	-	-	++	-
R. pickettii	11149	+++	+++	+++	++	+++	+++	+++
P. putida	10936	-	-	-	-	-	-	-
P. stutzeri	12262	+	+	-	-	-	-	-
P. stutzeri	10475	-	+	-	-	-	-	-
P. vesiculare	10900	-	-	+/-	-	+++	+	+++
S. spiritivorum	ATCC 33861	+++	++	+	++	-	+++	+++
B. ambifaria	11351	+++	+	-	++	-	+/-	+
B. andropogonis	1279	+++	-	-	+	-	++	+
B. andropogonis	2126	+++	++	-	++	-	+++	+++
B. caryphylli	2155	++	-	-	-	-	+	++
B. caryphylli	2156	+++	+/-	-	+/-	-	+/-	+++
B. dolosa	18941	-	+	-	-	-	-	-
B. dolosa	18942	-	+/-	-	-	-	-	-
B. gladioli	11626	+	-	-	-	-	-	-
B. gladioli	18113	+++	-	-	-	-	+/-	-
B.gladioli pv. alliicola	2121	+++	+	-	-	-	+/-	-
B. gladioli pv. alliicola	6877	+++	+/-	-	-	-	+/-	+/-
B.gladioli pv. gladioli	2216	+++	-	-	-	-	+	-

Appendix 3.6a (cont`d..): Colour intensity recordings for Carboxypeptidase substrates

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
B.gladioli pv. gladioli	6880	+++	-	-	-	-	+/-	-
B. glumae	1277	-	-	-	-	-	-	-
B. glumae	2196	-	+/-	-	-	-	-	-
B. phenazinium	2247	-	-	-	-	-	-	-
B. phenazinium	6868	+	+/-	-	-	-	-	-
P. apista	16408	+++	+/-	-	-	++	++	+++
P. norimberensis	13019	+++	+/-	-	-	++	++	+
P. norimberensis	16603	+++	+	-	-	++	++	+
P. pnomenusa	18087	+++	++	-	-	+	+++	++
P. pnomenusa	18817	+++	++	-	-	+	+	++
P. pulmonicola	18107	+++	++	+/-	-	+	+++	++
R. campinensis	19282	+++	+++	+++	+++	++	+++	+++
R. campinensis	19283	+++	+++	+++	+++	+	+++	+++
R. eutropha	1190	+++	+++	+++	+	+++	+++	+++
R. eutropha	1194	+++	+++	+++	++	+++	+++	+++
R. gilardii	3399	+++	+++	+++	+++	+++	+++	+++
R. gilardii	3400	+++	+++	++	++	+++	+++	+++
R. mannitolilytica	19090	+++	+++	+++	+++	+++	+++	+++
R. metallidurans	1195	+++	+++	+++	+++	++	+++	+++
R. metallidurans	19290	+++	+++	+++	++	++	+++	+++
R. paucula	3244	+++	+	-	+	+	+++	+++
R. paucula	3245	+++	++	-	-	+	+++	+++
R. pickettii	5942	+++	+++	+++	+++	+++	+++	+++
R. pickettii	6871	+++	+++	++	+	++	+++	+++
R. solanacearum	2291	-	-	-	-	-	+/-	-
R. solanacearum	2293	+++	+	-	-	-	+++	+++
R. taiwanensis	19425	+++	++	+/-	-	+	+++	+++
S. maltophilia	957	-	-	+/-	-	+/-	+	-
S. maltophilia	958	-	+/-	+	-	+/-	+/-	-

Key:
ben-L-ala, benzoyl-L-alanine; ben-L-glu, benzoyl-L-glutamic acid; ben-gly, benzoyl-glycine; ben-L-his, benzoyl-L-histidine; ben-DL-leu, benzoyl-DL-leucine; ben-DL-met, benzoyl-DL-methionine; ben-L-phe, benzoyl-L-phenylalanine.

Appendix 3.6b: Colour intensity recordings for Carboxypeptidase substrates (duplicate screen)

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
P. aeruginosa	2688	-	-	-	-	-	-	-
P. aeruginosa	2702	-	-	-	-	-	-	-
P. aeruginosa	2704	+++	+++	-	-	+	-	+++
P. aeruginosa	2706	-	-	-	-	-	-	-
P. aeruginosa	2715	-	-	-	-	-	-	-
P. aeruginosa	2720	-	-	-	-	-	-	-
P. aeruginosa	2737	-	-	-	-	-	-	-
P. aeruginosa	2739	-	-	-	-	-	-	-
P. aeruginosa	2741	-	-	-	-	-	-	-
P. aeruginosa	2742	-	-	-	-	-	-	-
P. aeruginosa	2749	-	-	-	-	-	-	-
P. aeruginosa	2772	-	-	-	-	-	-	-
P. aeruginosa	2775	+	-	-	-	-	-	-
P. aeruginosa	2776	+/-	-	-	-	-	-	-
P. aeruginosa	2778	++	-	-	+/-	-	-	-
P. aeruginosa	2779	-	-	-	-	-	-	-
P. aeruginosa	2780	-	-	-	-	-	-	-
P. aeruginosa	2781	+/-	-	-	-	-	-	-
P. aeruginosa	2782	-	-	-	-	-	-	-
P. aeruginosa	2783	-	-	-	-	-	-	-
P. aeruginosa	PS1	-	-	-	-	-	-	-
P. aeruginosa	PS2	-	-	-	-	-	-	-
P. aeruginosa	PS3	-	-	-	-	-	-	-
P. aeruginosa	PS4	-	-	-	-	-	-	-
P. aeruginosa	PS5	-	-	-	-	-	-	-
P. aeruginosa	PS6	-	-	-	-	-	-	-
P. aeruginosa	PS7	-	-	-	-	-	-	-
P. aeruginosa	PS8	-	-	-	-	-	-	-
P. aeruginosa	PS9	-	-	-	-	-	-	-
P. aeruginosa	PS10	-	-	-	-	-	-	-
P. aeruginosa	PS11	+/-	++	-	-	-	-	-
P. aeruginosa	PS12	-	-	-	-	-	-	-
P. aeruginosa	PS13	-	-	-	-	-	-	-
P. aeruginosa	PS14	-	-	-	-	-	-	-
P. aeruginosa	PS15	-	-	-	-	-	-	-
P. aeruginosa	PS16	-	-	-	-	-	-	-
P. aeruginosa	PS17	-	-	-	-	-	-	-
P. aeruginosa	PS18	-	-	-	-	-	-	-
P. aeruginosa	PS19	-	-	-	-	-	-	-
P. aeruginosa	PS20	-	-	-	-	-	-	-
P. aeruginosa	PS21	-	-	-	-	-	-	-
P. aeruginosa	PS22	-	-	-	-	-	-	-
P. aeruginosa	PS23	-	-	-	-	-	-	-
P. aeruginosa	PS24	-	-	-	-	-	-	-
P. aeruginosa	PS25	-	-	-	-	-	-	-
P. aeruginosa	PS26	-	-	-	-	-	-	-
P. aeruginosa	PS27	-	-	-	-	-	-	-
P. aeruginosa	PS28	-	-	-	-	-	-	-
P. aeruginosa	PS29	-	-	-	-	-	-	-
P. aeruginosa	PS30	-	-	-	-	-	-	-
P. aeruginosa	PS31	-	-	-	-	-	-	-

Appendix 3.6b (cont'd..): Colour intensity recordings for Carboxypeptidase substrates (duplicate screen)

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
P. aeruginosa	PS32	-	-	-	-	-	-	-
P. aeruginosa	PS33	-	-	+/-	-	-	-	-
P. aeruginosa	PS34	-	-	-	-	-	-	-
P. aeruginosa	PS35	-	-	-	-	-	-	-
P. aeruginosa	PS36	-	-	-	-	-	-	-
P. aeruginosa	PS37	-	-	-	-	-	-	-
P. aeruginosa	PS38	-	-	-	-	-	-	-
P. aeruginosa	PS39	-	-	-	-	-	-	-
P. aeruginosa	PS40	-	-	-	-	-	-	-
P. aeruginosa	PS41	-	-	-	-	-	-	-
P. aeruginosa	PS42	-	-	-	-	-	-	-
P. aeruginosa	PS43	-	-	-	-	-	-	-
P. aeruginosa	PS44	-	-	-	-	-	-	-
P. aeruginosa	PS45	-	-	-	-	-	-	-
P. aeruginosa	PS46	-	-	-	-	-	-	-
P. aeruginosa	PS47	-	-	-	-	-	-	-
P. aeruginosa	PS48	-	-	-	-	-	-	-
P. aeruginosa	PS49	-	-	-	-	-	-	-
P. aeruginosa	PS50	-	-	-	-	-	-	-
P. aeruginosa	PS51	-	-	-	-	-	-	-
P. aeruginosa	PS52	-	+/-	+	-	-	-	-
B. cepacia	LMG 1222	+++	+	-	+/-	-	++	+/-
B. cepacia	LMG 2161	+++	+	-	+	-	+++	+/-
B. cenocepacia	LMG 16654	+++	+	-	+/-	-	++	+/-
B. cenocepacia	LMG 16656	+++	+	-	+/-	-	++	+/-
B. cenocepacia	LMG 16659	+++	+	-	+	-	+++	-
B. cepacia	LMG 17997	+++	+/-	-	+	-	++	+/-
B. cepacia	LMG 18821	+	+/-	-	-	-	++	+
B. cenocepacia	LMG 18826	+++	+++	-	+/-	-	++	+/-
B. cenocepacia	LMG 18827	-	-	-	-	-	-	-
B. cenocepacia	LMG 18828	++	+/-	-	-	-	++	-
B. cenocepacia	LMG 18829	+++	+	-	+/-	-	++	+/-
B. cenocepacia	LMG 18830	+/-	-	-	-	-	-	-
B. cenocepacia	LMG 18832	+++	+	-	-	-	+	-
B. cenocepacia	LMG 18863	+++	+	-	+	-	+/-	-
B. multivorans	LMG 13010	+++	+++	+	+/-	-	+++	-
B. multivorans	LMG 16660	+++	+	-	+/-	-	++	-
B. multivorans	LMG 16665	+++	+	-	+/-	-	++	+/-
B. multivorans	LMG 17588	+++	+	-	+/-	-	+	-
B. multivorans	LMG 18822	+++	+	-	+/-	-	+	-
B. multivorans	LMG 18823	+++	+/-	-	-	-	+/-	-
B. multivorans	LMG 18824	+++	+	-	+/-	-	+	-
B. multivorans	LMG 18825	+++	+	-	+/-	-	++	-
B. stabilis	LMG 14086	+++	++	-	+/-	+/-	+++	+
B. stabilis	LMG 14294	-	-	-	-	-	-	-
B. stabilis	LMG 18870	+++	+	-	++	-	++	+/-
B. stabilis	LMG 18888	+++	+++	-	+++	+/-	+++	+++

Appendix 3.6b (cont'd..): Colour intensity recordings for Carboxypeptidase substrates (duplicate screen)

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
B. vietnamiensis	LMG 10929	+++	++	-	+++	-	+	+/-
B. vietnamiensis	LMG 16232	+/-	-	-	+/-	-	-	-
B. vietnamiensis	LMG 18835	+/-	-	-	+/-	-	-	-
B. vietnamiensis	LMG 18836	+++	+/-	-	+++	-	-	-
A. baumannii	ATCC 1960(	+++	+++	+	+++	-	+++	+++
A. calcoaceticus	7844	+++	+++	+/-	+++	-	+++	+++
A. haemolyticus	12155	+++	+++	-	+++	-	+/-	+++
A. johnsonii	10308	+++	+	-	++	-	++	-
B. vesicularis	ATCC 1142(	+++	+++	+	+++	+/-	+++	+++
R. pickettii	11149	+++	++	+++	+/-	+++	+++	+++
C. meningosepticum	ATCC 13253	-	-	-	-	-	-	-
M. nonliquefaciens	10464	-	-	-	-	-	-	-
M. osloensis	10465	-	-	-	-	-	+	+
M. urethralis	11010	++	+/-	-	+	-	++	-
O. urethralis	11999	+++	+++	++	+++	-	++	+++
P. acidovorans	10683	-	-	-	-	-	-	-
P. aeruginosa	6749	-	-	-	-	-	-	-
P. aeruginosa	10332	-	-	-	-	-	-	-
P. alcaligenes	10367	+++	+++	+	+++	-	+++	++
P. pseudoalcaligenes	10860	-	-	-	-	-	-	-
P. diminuta	8545	+++	+++	+++	+++	+++	++	++
P. fluorescens	10754	+	++	+/-	-	+/-	+	+/-
P. fluorescens	10392	-	-	-	-	-	-	-
P. fluorescens	3756	-	-	-	-	-	-	-
P. fluorescens	10038	-	-	++	-	-	-	-
P. fluorescens	10688	+/-	-	+/-	+/-	-	++	+/-
P. fluorescens	9428	-	-	+++	-	-	-	-
P. fragi	NCIMB 898	+	++	++	-	-	-	-
P. maltophilia	10257	+/-	+	+/-	-	+/-	+/-	-
P. paucimobilis	11030	+++	+++	-	-	-	+/-	-
R. pickettii	11149	+++	+++	+++	++	+++	+++	+++
P. putida	10936	-	+/-	-	-	-	-	-
P. stutzeri	12262	-	+/-	-	-	-	-	-
P. stutzeri	10475	+++	+++	-	-	-	+/-	-
P. vesiculare	10900	-	-	+/-	-	+	+/-	+
S. spiritivorum	ATCC 33861	+++	++	+	+++	-	++	+++
B. ambifaria	11351	+++	++	-	+++	-	+	+
B. andropogonis	1279	+++	-	-	+	-	+	-
B. andropogonis	2126	++	-	-	-	-	+	-
B. caryphylli	2155	++	+/-	-	+++	-	++	+++
B. caryphylli	2156	+++	+++	-	-	-	-	+/-
B. dolosa	18941	+	+++	-	-	-	+/-	-
B. dolosa	18942	-	-	-	-	-	-	-
B. gladioli	11626	+++	+	-	-	-	-	-
B. gladioli	18113	+++	-	-	-	-	+/-	-
B.gladioli pv. alliicola	2121	+++	+	-	-	-	-	-
B. gladioli pv. alliicola	6877	+++	-	-	+/-	-	+/-	-
B.gladioli pv. gladioli	2216	+++	-	-	-	-	-	-

Appendix 3.6b (cont`d..): Colour intensity recordings for Carboxypeptidase substrates (duplicate screen)

		Substrate						
Strain	**Reference**	ben-gly	ben-L-ala	ben-L-glu	ben-L-his	ben-DL-leu	ben-DL-met	ben-L-phe
B.gladioli pv. gladioli	6880	++	+/-	-	-	-	+/-	-
B. glumae	1277	-	-	-	-	-	-	-
B. glumae	2196	-	-	-	+/-	-	-	-
B. phenazinium	2247	+	+++	-	-	-	-	-
B. phenazinium	6868	+/-	++	-	-	-	-	-
P. apista	16408	+++	++	-	-	+	+++	++
P. norimberensis	13019	++	-	-	-	+++	+++	+/-
P. norimberensis	16603	++	+/-	-	-	++	++	+++
P. pnomenusa	18087	+++	+++	-	-	+	++	+
P. pnomenusa	18817	+++	++	-	-	++	+++	++
P. pulmonicola	18107	+++	+++	-	-	++	+++	++
R. campinensis	19282	+++	++	-	+/-	+/-	+++	+++
R. campinensis	19283	+++	+++	+++	+++	+	+++	+++
R. eutropha	1190	+++	+++	+++	+++	+++	+++	+++
R. eutropha	1194	+++	+++	+++	+++	+++	+++	+++
R. gilardii	3399	+++	+++	+++	+++	+++	+++	+++
R. gilardii	3400	+++	+++	+++	+++	+++	+++	+++
R. mannitolilytica	19090	+++	+++	+++	+++	+++	+++	+++
R. metallidurans	1195	+++	+++	++	++	+	+++	+++
R. metallidurans	19290	+++	+++	+++	+++	++	+++	+++
R. paucula	3244	+++	+++	+++	+++	+	+++	+++
R. paucula	3245	+++	+++	-	+/-	++	+++	+++
R. pickettii	5942	+++	++	+++	++	++	+++	+++
R. pickettii	6871	+++	++	+++	-	++	+++	+++
R. solanacearum	2291	++	+/-	-	+/-	-	++	+/-
R. solanacearum	2293	+++	++	-	+++	-	+++	+++
R. taiwanensis	19425	+++	++	+/-	-	+	+++	+++
S. maltophilia	957	-	+	++	-	+	++	-
S. maltophilia	958	-	+/-	+	-	+/-	+/-	-

Key:
ben-L-ala, benzoyl-L-alanine; ben-L-glu, benzoyl-L-glutamic acid; ben-gly, benzoyl-glycine; ben-L-his, benzoyl-L-histidine; ben-DL-leu, benzoyl-DL-leucine; ben-DL-met, benzoyl-DL-methionine; ben-L-phe, benzoyl-L-phenylalanine.

Appendix 3.7: Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc IV	CEP0717	6934	6586	1220	1553	333	-494	16654	1526
Bcc III-A	CEP0506	2677	3672	-43	101	144	-479	-83	-22
Bcc V	CEP0126	2124	7807	-42	1266	1308	-278	-61	9
Bcc III-A	CEP0824	592	10258	751	76	-675	-507	22	-49
Bcc I	CEP0060	2686	19487	348	10573	10225	-250	13062	14091
Bcc IV	CEP0469	4694	11500	1337	806	-531	-516	7346	195
Bcc V	CEP0999	976	7035	-70	1724	1794	-354	-61	55
Bcc III-B	CEP0762	2967	6	315	64	-251	-510	-67	12
Bcc III-A	C4708	2878	7044	-33	33	66	-531	-83	-22
Bcc III-B	CEP0562	-85	3	-34	19	53	-504	-61	9287
Bcc IV	CEP0112	3894	10499	650	1318	668	-470	24440	2414
Bcc III-B	CEP0519	3831	18282	317	2097	1780	-158	-55	19231
Bcc VI	CEP0028	2222	9394	626	538	-88	-464	-73	943
Bcc II	C8467	2854	8277	-71	564	635	-479	-49	-43
Bcc III-A	CEP0198	3058	8423	-45	665	710	-415	-73	2197
Bcc II	C9281	1550	10776	-45	696	741	-388	-64	-15
S. maltophilia	C4775	3852	235	16212	275	-15937	-559	5076	4255
Bcc III-A	CEP0444	2655	3958	235	131	-104	-544	-49	64
Bcc II	CEP1006	5024	7212	-49	36	85	-516	-67	-55
Bcc I	CEP0533	1877	10627	396	2024	1628	-394	-64	5677
Bcc III-A	CEP0507	6281	12415	873	806	-67	-479	-79	1761
Bcc II	CEP0773	1828	5799	-70	161	231	-522	-61	-40
Bcc V	CEP1110	2735	10508	-61	470	531	-467	-64	12
Bcc III-B	CEP0961	2170	14586	202	241	39	-555	-61	76
Bcc V	CEP0974	2192	9098	-55	73	128	-500	-80	-55
A. xyosoxidans	CEP0688	-48	-3	-48	15	63	-531	-64	-73
Bcc VI	CEP0027	3198	6202	815	903	88	-293	-64	-30
Bcc V	CEP0339	1117	3	-43	107	150	-485	-70	-22
Bcc III-B	CEP0136	7056	504	190	79	-111	-501	-77	222
Bcc III-A	CEP0942	4700	8524	739	2331	1592	-278	-83	12702
Bcc V	CEP0143	1019	5085	-58	222	280	-476	-73	-65
Bcc V	CEP0505	4258	265	-36	15	51	-501	-82	-46
Bcc III-A	FC0427	5240	1071	522	110	-412	-559	-76	-43
Bcc II	CEP0181	5799	8482	-42	314	356	-464	-70	851
Bcc III-B	CEP0750	5789	4615	419	146	-273	-546	-82	-9
Bcc III-A	CEP0663	5444	10288	445	134	-311	-509	-67	280
S. maltophilia	C5001	8338	766	12818	70	-12748	-525	6108	10264
P. sputorum	CEP0687	-88	-18	-67	27	94	-544	-64	-37
Bcc II	C4356	3824	6595	-37	40	77	-522	-97	-55
Bcc III-A	CEP0242	3189	8943	596	583	-13	-461	-94	131
Bcc II	CEP0978	1520	6708	-54	192	246	-436	-73	-25
P. sputorum	C4964	-27	-12	-74	0	74	-507	-34	-19
Bcc III-A	C6279	5750	3476	574	45	-529	-479	-58	-52
Bcc VII	CEP0516	2594	9458	1410	1150	-260	-317	-70	21019
Bcc III-A	CEP0755	4694	3818	1083	220	-863	-497	-73	42
Bcc III-B	CEP1114	4364	6961	-86	149	235	-449	-85	-49
Bcc II	CEP0938	4936	8389	-77	378	455	-455	-103	-31
Bcc III-B	CEP0984	2704	11183	226	1605	1379	-314	-64	9025
Acinetobacter sp	CEP0684	-61	12	-61	33	94	-442	-82	-55

Appendix 3.7 (cont'd..): Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc IV	CEP0194	13221	7230	1328	330	-998	-445	-49	-12
Bcc III-B	CEP0499	2863	14552	439	879	440	-387	-58	51
Bcc III-A	CEP0715	1495	1654	-80	30	110	-421	-76	-43
Bcc III-A	CEP0749	1495	49	192	12	-180	-446	-67	-58
Bcc II	C7273	3473	6660	-67	137	204	-458	-58	-49
Bcc V	CEP0086	586	7105	-61	873	934	-299	-76	-21
Bcc IV	CEP0176	5158	8207	2209	167	-2042	-425	-76	-15
Bcc III-A	C4872	10062	2069	268	82	-186	-437	-92	55
Bcc III-A	CEP0236	2152	11928	-71	1141	1212	-262	-91	13908
Bcc V	CEP0480	961	6550	-64	55	119	-412	-79	-43
Bcc III-A	CEP0787	1600	5332	-86	49	135	-474	-30	-21
Bcc I	CEP0957	3388	15196	1767	888	-879	-309	6733	302
Bcc IV	CEP1025	2890	12864	2280	492	-1788	-406	5424	1791
Bcc I	FC0449	3290	9272	699	1254	555	-296	12730	6897
Bcc V	FC0464	812	6867	-33	342	375	-418	-64	-12
Bcc V	CEP0213	1175	10704	-70	164	234	-424	-70	-49
Bcc IV	CEP0500	6357	6809	1948	601	-1347	-415	8542	92
Bcc III-A	CEP0168	2845	8723	-45	1117	1162	-49	-76	1953
Bcc IV	CEP0711	4002	8064	1923	351	-1572	-184	2057	16
Bcc VI	CEP0735	2512	6776	2176	345	-1831	-336	-73	-43
Bcc I	CEP0069	1801	11390	779	3427	2648	-293	-88	12013
Bcc II	C9346	3641	11628	-61	186	247	-500	-61	-43
Bcc IV	CEP0952	4545	17024	3403	894	-2509	-467	5347	174
Bcc IV	CEP1075	2517	15150	1398	775	-623	-528	6876	2020
Bcc II	CEP0503	1285	788	-55	34	89	-550	-70	-58
Bcc III-A	CEP0211	2640	7956	-82	1496	1578	-336	-52	6303
Bcc II	CEP0493	2344	5842	-40	211	251	-540	-55	-52
Bcc V	D0439	879	565	-73	-6	67	-595	-55	-46
Bcc III-A	CEP0209	2537	9779	-64	2222	2286	-177	-76	19219
Bcc I	CEP0843	3174	5549	348	1672	1324	-309	8839	6928
Bcc II	C7363	3385	8747	-64	473	537	-437	-58	-21
Bcc III-A	C7837	2494	1465	327	12	-315	-592	-73	-52
Bcc II	C8982	2649	1261	-58	42	100	-528	-85	-61
Bcc I	CEP0067	1797	1206	873	632	-241	-461	-73	4483
Bcc V	CEP0741	1660	8762	-48	144	192	-567	-73	-33
Bcc III-B	C7946	3326	15690	391	2173	1782	-180	-64	9763
Bcc II	CEP1018	2261	2659	-46	159	205	-528	-79	-46
Bcc VI	CEP1007	1081	61	818	134	-684	-565	-67	-9
Bcc VI	CEP1014	1926	7713	1075	299	-776	-525	-73	-34
Bcc VI	CEP0766	1429	7584	1059	544	-515	-543	-67	12
R. pickettii	CEP0096	-73	4896	-49	21	70	-565	-58	-33
A. denitrifications	CEP0184	80	-24	-58	33	91	-604	-61	-28
A. denitrifications	CEP0183	211	-18	-55	33	88	-556	-58	-70
A. faecalis	CEP0864	-110	-33	-80	52	132	-546	-55	-34
A. denitrifications	C9774	296	-33	-39	24	63	-586	-52	-67
S. maltophilia	CEP0406	2554	333	16691	168	-16523	-623	6333	5521
S. maltophilia	CEP0272	10338	470	22194	27	-22167	-705	0	415
P. apista	C8757	248	46	195	31	-164	125	-3	-12
P. pnomenusa	C7351	36	68	37	33	-4	260	-15	-6

Appendix 3.7 (cont`d..): Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc VI	CEP1010	2185	10108	1395	250	-1145	317	6	18
Bcc VI	CEP1008	1083	79	1221	152	-1069	266	-21	55
Bcc V	CEP0649	3284	134	3	70	67	272	3	15
R. pickettii	C6529	174	216	15	12	-3	235	-18	21
S. maltophilia	FC0827	7075	781	16548	149	-16399	217	6629	9935
Bcc I	CEP1030	2905	214	418	122	-296	226	61	9309
P. pnomenusa	C8029	61	61	19	15	-4	244	-9	30
R. pickettii	C8770	70	6266	10	27	17	247	-15	-19
Bcc VIII	FC0972	2402	13215	674	65	-609	256	27	36
Bcc VIII	FC0970	2134	10181	677	65	-612	210	9	9
Bcc III-B	CEP0616	4691	1349	418	119	-299	229	6	33
Bcc VIII	FC0973	1456	9919	458	27	-431	226	-6	6
Bcc VI	FC0346	1749	8612	1441	335	-1106	266	-6	18
Bcc VII	FC0662	1719	4562	470	204	-266	180	-21	2799
Bcc V	CEP0639	659	10084	-12	610	622	268	3	88
Bcc I	CEP1032	3018	9757	718	5485	4767	546	14601	14250
A. xylosoxidans	D0423	717	52	0	34	34	146	-9	9
Bcc III-A	CEP0498	5662	6113	196	88	-108	168	30	24
Bcc III-A	CEP0655	2124	8988	1153	280	-873	146	-12	332
A. xylosoxidans	FC0815	247	265	10396	24	-10372	82	-12	0
Bcc IV	C7838	8366	3083	964	88	-876	103	28	70
Bcc I	C8729	1910	1590	296	110	-186	186	6	415
Bcc III-A	CEP0880	1547	7425	373	129	-244	152	3	88
Bcc I	CEP0101	870	1245	283	229	-54	183	-15	174
Bcc III-A	CEP0785	5127	226	137	82	-55	143	-18	6
Bcc V	CEP0041	406	3375	31	40	9	180	-3	-9
Bcc II	C5394	1483	5487	-3	186	189	250	0	24
Bcc IV	CEP0185	2631	11509	1272	332	-940	217	6321	125
Bcc III-A	CEP1199	2030	91	299	49	-250	186	3	-52
Bcc II	C5449	2326	2921	-6	67	73	210	-6	0
Bcc V	CEP0196	940	7486	-12	213	225	238	-3	55
Bcc III-A	CEP0186	2964	13380	3	891	888	412	0	4099
Bcc IV	CEP1081	3058	17158	1746	692	-1054	256	6583	839
Bcc I	CEP0070	8372	4072	689	1379	690	388	-25	24935
Bcc I	CEP0072	1444	10612	384	2450	2066	351	40	17253
Bcc III-B	CEP0117	6581	8479	287	1437	1150	628	6	7331
Bcc I	CEP0972	1639	7047	436	757	321	354	9434	693
R. pickettii	C1139	40	6874	18	24	6	208	-9	-3
Bcc III-B	CEP1111	7380	6314	147	1623	1476	391	-6	485
A. xylosoxidans	C2302	58	67	13	24	11	168	15	-12
Bcc II	CEP0965	3101	5417	-7	158	165	137	-9	12
Bcc III-A	CEP0565	3851	3687	369	427	58	226	-12	326
Bcc V	CEP0087	613	7892	13	445	432	186	12	58
Bcc III-A	CEP1091	6860	14552	1166	119	-1047	113	3171	3
Bcc IV	CEP0118	4526	17055	1658	1223	-435	220	2033	1163
Bcc V	C8766	3760	2341	19	70	51	159	9	55
Bcc III-B	CEP1113	5833	7178	412	73	-339	192	3	9
Bcc VI	CEP0026	1950	7514	934	634	-300	345	6	95
Bcc II	CEP0485	1297	5203	-27	88	115	226	15	-18

Appendix 3.7 (cont'd..): Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc I	CEP0081	1755	8710	516	1071	555	253	0	15596
Bcc I	CEP0068	1279	6696	473	2191	1718	341	3	19121
Bcc III-B	CEP0114	2002	5170	614	122	-492	107	9	418
Bcc IV	CEP0929	2958	7862	2146	168	-1978	128	2222	104
Bcc III-B	CEP0139	4670	125	827	64	-763	103	3	52
Bcc II	C9172	1370	6827	24	458	434	247	-30	52
Bcc III-A	C6749	2457	5554	-6	595	601	235	3	3232
Bcc II	CEP1116	1956	6058	34	67	33	104	3	18
Bcc I	CEP0934	1700	12998	431	3564	3133	1059	20159	29192
Bcc III-A	CEP0869	1416	7169	216	140	-76	161	9	229
Bcc III-A	CEP1079	3113	8335	506	303	-203	192	9	192
A. xylosoxidans	CEP1094	61	64	7	-3	-10	155	0	-12
Bcc III-A	CEP0951	2164	94	440	18	-422	138	-24	3
Bcc IV	C6061	5784	134	757	24	-733	61	-24	3
Bcc II	CEP0786	1746	2924	25	39	14	61	55	98
Bcc IV	CEP0945	4156	10645	2741	308	-2433	129	4551	107
Bcc III-A	CEP0408	2707	125	422	30	-392	43	-6	64
Bcc I	CEP1190	2585	5271	848	1751	903	235	-3	14289
Bcc IX	FC0433	1629	13612	843	1066	223	324	-15	10026
Bcc VI	CEP1012	2240	4230	1614	342	-1272	122	-33	24
Bcc III-B	1114	9224	146	424	125	-299	165	9	116
Bcc II	CEP1235	6253	4785	25	324	299	296	-6	55
Bcc III-A	CEP0591	7770	116	293	15	-278	156	30	15
Bcc IV	FC0777	1312	4767	2218	248	-1970	159	-9	241
Bcc VIII	FC0969	1983	10432	662	37	-625	207	-9	21
A. xylosoxidans	FC0349	24	43	16	39	23	208	9	24
A. denitrifications	CEP0092	580	58	22	27	5	95	21	-12
Bcc III-B	CEP0769	4996	238	641	101	-540	98	0	134
Bcc II	CEP0686	2802	7941	28	94	66	58	3	18
Bcc III-B	FC0378	4944	4203	315	171	-144	241	116	394
H_2O		119	70	9	15	6	-46	-12	-16
H_2O		21	37	25	18	-7	131	6	-6
H_2O		39	61	16	21	5	110	18	-3
H_2O		52	64	9	30	21	101	6	12
H_2O		24	33	28	6	-22	113	-21	-9
E. coli		5442	82	10019	21	-9998	82	1215	1084
E. cloacae		1325	52	6742	26134	19392	168	1215	104
Bcc V	FC0465	2048	22853	-46	1157	1203	52	-9	830
Bcc I	CEP0615	5091	27923	2258	290	-1968	-16	3	12449
Bcc II	FC0357	13295	4053	-46	714	760	101	85	-6
Bcc VI	FC0353	8250	7258	-34	428	462	36	-9	25
Bcc II	FC0102	6284	18920	-43	601	644	-22	-24	-3
Bcc II	CEP0691	10194	16496	-46	543	589	-12	-6	-3
Bcc III-A	CEP1161	14512	21468	3116	3091	-25	415	-15	5109
Bcc III-A	FC0120	22316	3019	3174	0	-3174	-55	-13	35186
Bcc II	D0156	7932	24737	-52	183	235	-28	12	12
Bcc II	D0297	4214	15596	-46	387	433	-15	0	27
Bcc VI	CEP1013	5512	18846	3446	1456	-1990	109	-18	28
Bcc IV	FC0362	9559	20097	5625	2964	-2661	122	34789	10936

Appendix 3.7 (cont'd..): Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc VIII	FC0961	13310	27022	1926	64	-1862	4	-3	-9
Bcc VIII	FC0964	9336	23760	2301	58	-2243	-40	6	16
Bcc VIII	FC0965	3266	27703	3461	-51	-3512	-46	-12	6
Bcc III-B	CEP0628	9287	21129	1514	3085	1571	479	-6	27602
Bcc III-A	CEP0768	11326	28161	266	-4	-270	-21	15	9
Bcc II	CEP0699	6370	12569	-55	852	907	48	-15	58
Bcc III-B	CEP0986	6742	15681	-58	323	381	9	-12	171
Bcc II	CEP0455	5759	2216	708	-28	-736	-40	-33	3
Bcc V	C9710	4743	26168	-40	152	192	-37	-3	351
Bcc III-A	CEP0215	9052	23138	4822	412	-4410	64	22546	25801
Bcc II	C7637	6290	12287	-37	393	430	-31	-3	91
Bcc V	C9287	3055	26919	-37	1932	1969	89	21	1034
Bcc III-B	CEP0187	12989	9339	760	1710	950	345	-9	4398
Bcc IV	CEP0103	6672	18123	10493	1340	-9153	12	16578	13557
Bcc II	C8681	8848	21129	-21	1538	1559	61	6	24
Bcc V	C9371	4340	15294	-10	82	92	-28	-9	28
Bcc IV	CEP0235	6232	25377	4786	568	-4218	-15	2228	4340
Bcc I	CEP0240	2060	21190	2329	1383	-946	34	-15	709
Bcc VII	CEP0102	11430	8924	3372	1963	-1409	184	-18	9874
Bcc II	C5275	6836	15300	-52	598	650	-13	-9	3
Bcc I	CEP0073	5786	27694	1486	4511	3025	141	-97	32767
Bcc V	CEP0084	20378	30383	3300	223	-3077	-28	21	1188
Bcc VII	CEP0617	6363	17402	2008	687	-1321	12	-15	22181
Bcc II	CEP0169	11866	21477	-40	821	861	37	-21	31
Bcc V	C8952	2643	29687	-40	412	452	79	18	232
Bcc III-A	CEP0146	12059	26421	-49	1789	1838	253	3	20656
Bcc I	CEP0076	2689	13716	1114	16773	15659	1670	6	8872
Bcc V	CEP0192	3556	15318	-64	803	867	18	-12	379
Bcc III-A	CEP0606	10829	17518	2054	4486	2432	595	-9	12419
Bcc V	CEP0047	2280	16783	-46	690	736	36	-27	718
R. pickettii	CEP0488	-15	14845	-58	-58	0	-22	-6	-37
Bcc III-A	CEP0931	8567	15971	2725	1343	-1382	64	-24	5659
Bcc IV	CEP0404	7547	24456	6956	1355	-5601	12	989	702
Bcc II	C7263	5545	16450	-55	1050	1105	43	-15	28
Bcc I	C8536	4773	5091	1087	217	-870	-28	6	11713
Bcc V	CEP0233	2906	30572	-39	1993	2032	100	-34	1117
Bcc IV	CEP0142	8875	17635	6531	1132	-5399	55	-15	3208
Bcc II	D0056	16237	15104	-43	778	821	18	-12	19
Bcc I	CEP0074	5023	21279	1361	7544	6183	305	-3	9745
Bcc II	CEP0243	7016	12910	-30	809	839	79	-37	19
Bcc II	D0155	6458	13130	-25	354	379	-31	-24	-22
Bcc V	FC0373	6382	4044	-19	559	578	55	-18	-12
Bcc I	CEP1140	9574	15775	1770	8649	6879	531	6297	14470
Bcc V	D0278	26540	8887	-15	668	683	36	24	446
Bcc II	C7329	3705	19716	-45	1868	1913	88	-9	37
Bcc V	FC0622	2921	18386	-46	467	513	-12	0	550
Bcc III-A	CEP001	7124	10759	-37	9	46	-16	-34	16
Bcc V	CEP1236	1484	20326	67	1029	962	39	-10	916
Bcc II	CEP0601	8976	28426	-39	1272	1311	64	40	43

Appendix 3.7 (cont'd..): Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc II	CEP0108	7935	21270	-64	506	570	-16	25	7
Bcc V	CEP0175	3299	22164	-43	2087	2130	116	-21	483
Bcc I	CEP0990	10441	20476	907	2093	1186	198	-15	3995
Bcc II	CEP1000	11356	9416	-58	614	672	34	-39	16
Bcc V	CEP0865	4978	12543	-37	1478	1515	100	-15	400
Bcc IV	CEP1064	4471	21895	1315	1957	642	7349	6370	1654
Bcc VII	CEP0958	3796	10459	904	1691	787	110	-9	3287
Bcc I	CEP0834	4389	17100	790	2793	2003	122	3565	11708
Bcc III-B	CEP0054	8103	13026	552	199	-353	132	-27	2060
Bcc III-A	CEP0156	2011	26983	-61	790	851	43	-30	614
Bcc IV	CEP0846	9503	19374	5241	4648	-593	95	30141	4035
Bcc IV	CEP0851	10118	18117	6397	610	-5787	3	29717	1441
A. xylosoxidans	C3968	3068	20400	-33	1001	1034	80	-6	-9
Bcc V	CEP0982	21	73	-46	-45	1	-46	-24	598
Bcc VI	CEP0873	4432	20958	2582	1127	-1455	116	-18	28
Bcc IV	FC0473	35937	23607	5854	6	-5848	-52	-18	-18
Bcc VI	CEP0021	5717	21068	2970	3672	702	412	140	67
Bcc IV	CEP0710	8088	25316	3614	531	-3083	1069	5271	611
Bcc III-B	CEP1067	8595	25612	1310	4130	2820	665	-21	8911
Bcc VII	CEP0996	20385	9021	436	1435	999	119	9	4450
Bcc VII	CEP1232	4032	17830	876	1141	265	55	-21	21501
Bcc I	FC0460	20701	4978	3312	-6	-3318	-22	-42	33
Bcc VI	CEP0743	2418	155	3229	293	-2936	6	-27	355
Bcc I	FC0660	8900	19136	1364	4853	3489	3473	3	31795
Bcc VI	CEP1011	8006	17775	3116	2746	-370	332	-31	73
Bcc II	CEP1129	3052	24178	-25	941	966	43	-33	-16
Bcc III-A	FC1057	6220	342	2579	106	-2473	-27	6391	9
Bcc I	FC0457	10518	7297	1648	-27	-1675	-58	-3	-25
Bcc I	CEP1132	6519	32446	1264	8464	7200	357	27651	27407
Bcc V	CEP1224	3555	11735	-52	3702	3754	344	58	9665
Bcc V	SQ004C	1795	23769	-46	656	702	400	6	1166
Bcc II	CEP0630	4664	22325	-40	333	373	3	-9	122
Bcc VIII	FC0974	3122	25304	1374	-9	-1383	-74	-12	33
Bcc III-B	CEP1119	9015	30334	1468	3732	2264	888	-30	35134
Bcc III-A	CEP0703	6886	9861	58	290	232	73	31	635
Bcc V	CEP0706	14583	17338	74	73	-1	46	34	73
Bcc VII	FC0881	5399	29821	4389	5329	940	766	104	37582
Bcc IV	FC0772	7823	24993	3690	2988	-702	195	39431	3156
Bcc II	CEP1129	15721	13203	61	675	614	71	42	52
Bcc V	D0121	2441	705	74	198	124	34	33	284
Bcc IV	CEP0059	29656	9330	4691	2557	-2134	216	7498	4773
Bcc V	CEP0255	6501	18470	39	4654	4615	406	28	3427
Bcc I	FC1104	3135	21956	1230	9867	8637	748	21	40018
Bcc VIII	FC0962	3061	19127	1742	30	-1712	34	15	49
Bcc VI	FC0380	6373	26110	6858	4175	-2683	497	9	82
Bcc VIII	FC0963	7679	27855	3418	61	-3357	64	21	15
Bcc II	D0445	5372	6300	74	855	781	146	3	88
Bcc III-A	CEP0702	6611	1947	49	165	116	67	15	1285
Bcc III-A	CEP0300	15864	7438	811	73	-738	52	3	39

Appendix 3.7 (cont'd..): Increases in fluorescence caused by various bacteria from Collection B due to hydrolysis of fluorogenic substrates after 18 hr incubation

		Substrate							
Strain	**Reference**	MU-phos	MU-pal	MU-rib	MU-xyl	pyr-AMC	MU-ß-fuc	MU-α-gal	MU-ß-glu
Bcc III-B	FC0372	16106	21699	1547	851	-696	171	28	16182
Bcc II	FC0898	6074	131	49	100	51	61	19	52
Bcc VIII	FC0967	5213	20646	1980	103	-1877	49	13	46
Bcc III-A	FC0506	7783	19148	58	3815	3757	482	6	29412
Bcc VII	FC0623	9434	20079	2380	6214	3834	427	15	38794
Bcc VII	FC0882	3839	15669	3970	491	-3479	110	16	11927
Bcc III-B	FC0802	6574	22472	336	1901	1565	293	21	4858
Bcc IV	FC0503	15400	21987	1181	82	-1099	22	70	64
Bcc III-B	FC0499	9244	161	775	692	-83	77	24	372
Bcc IX	FC0451	3733	25622	67	4560	4493	565	13	5695
Bcc V	FC0463	2359	21358	68	4016	3948	357	16	1227
Bcc III-A	CEP0635	9543	9699	1217	1062	-155	195	-12	5939
Bcc III-B	CEP1184	8427	128	1178	88	-1090	58	-9	10908
Bcc I	FC1107	2698	14793	1007	7608	6601	351	6	12779
Bcc IV	FC0778	11933	11936	5710	928	-4782	49	1511	259
Bcc VIII	FC0968	4887	14732	1867	104	-1763	34	9	46
Bcc III-B	CEP1139	10398	25200	1733	6592	4859	1108	-28	12205
Bcc VIII	FC0976	4916	10368	4536	577	-3959	46	28	125
Bcc I	FC1108	693	7990	876	726	-150	101	18	7755
Bcc I	CEP1151	4947	15824	1056	14683	13627	727	30999	35662
Bcc VII	FC0767	3068	12293	1303	1794	491	244	27	23562
Bcc I	FC0649	3668	36123	2026	2927	901	168	16	201
Bcc V	FC0659	3223	17433	40	1245	1205	146	0	739
H_2O		40	64	46	48	2	33	12	12
H_2O		43	55	45	52	7	49	-9	21
H_2O		34	52	45	46	1	34	-9	3
H_2O		31	49	34	61	27	33	16	-9
H_2O		52	64	33	52	19	46	25	-40
E. coli		8170	128	16041	70	-15971	161	1777	214
Enterobacter cloacae		4010	134	16441	29574	13133	665	3474	2237

Key:

Bcc I, *B. cepacia*; Bcc II, *B. multivorans*; Bcc III, *B. cenocepacia*; Bcc IV, *B. stabilis*; Bcc V, *B. vietnamiensis*; Bcc VI, *B. dolosa*; Bcc VII, *B. ambifaria*; Bcc VIII, *B. anthina*; Bcc IX, *B. pyrrocinia*

MU-pal, 4-methylumbelliferyl-palmitate; MU-rib, 4-methylumbelliferyl-ß-D-riboside; MU-xyl, 4-methylumbelliferyl-ß-D-xyloside; pyr-AMC, L-pyroglutamyl-7-amido-4-methylcoumarin ; MU-ß-fuc, 4-methylumbelliferyl-ß-D-fucoside; MU-α-gal, 4-methylumbelliferyl-α-D-galactoside; MU-ß-glu, 4-methylumbelliferyl-ß-D-glucoside

Colour intensity recordings of

Appendix 3.8: Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc IV	CEP0717	++	-	+	+
Bcc III-A	CEP0506	+/-	-	-	+
Bcc V	CEP0126	+	-	-	+
Bcc III-A	CEP0824	-	-	-	+
Bcc I	CEP0060	+	-	-	+
Bcc IV	CEP0469	+	-	+	+
Bcc V	CEP0999	-	-	-	+
Bcc III-B	CEP0762	+	-	-	+
Bcc III-A	C4708	+	-	-	+
Bcc III-B	CEP0562	+/-	+	++	+
Bcc IV	CEP0112	++	-	+/-	+
Bcc III-B	CEP0519	+	-	+	+
Bcc VI	CEP0028	+	-	-	+
Bcc II	C8467	++	-	-	+
Bcc III-A	CEP0198	+	-	-	+
Bcc II	C9281	+/-	-	-	+
S. maltophilia	C4775	+	+++	+	+
Bcc III-A	CEP0444	+	-	-	+
Bcc II	CEP1006	-	-	-	+
Bcc I	CEP0533	+	-	-	+
Bcc III-A	CEP0507	++	+/-	+	+
Bcc II	CEP0773	+	-	-	+
Bcc V	CEP1110	+	-	-	+
Bcc III-B	CEP0961	+	-	-	+
Bcc V	CEP0974	+/-	-	-	+
A. xyosoxidans	CEP0688	-	-	-	+
Bcc VI	CEP0027	+	-	-	+
Bcc V	CEP0339	+	-	-	+
Bcc III-B	CEP0136	++	-	-	+
Bcc III-A	CEP0942	+	+/-	+/-	+
Bcc V	CEP0143	+	-	-	+
Bcc V	CEP0505	+	-	-	+
Bcc III-A	FC0427	+/-	-	-	+
Bcc II	CEP0181	++	-	-	+
Bcc III-B	CEP0750	++	+/-	-	+
Bcc III-A	CEP0663	++	-	-	+
S. maltophilia	C5001	++	+	++	+
P. sputorum	CEP0687	-	-	-	+
Bcc II	C4356	+/-	-	-	+
Bcc III-A	CEP0242	+	-	-	+
Bcc II	CEP0978	+/-	-	-	+
P. sputorum	C4964	-	-	-	+
Bcc III-A	C6279	+	-	-	+
Bcc VII	CEP0516	+	-	-	+
Bcc III-A	CEP0755	+/-	-	-	+
Bcc III-B	CEP1114	++	-	-	+
Bcc II	CEP0938	+	-	-	+
Bcc III-B	CEP0984	+	-	-	+
Acinetobacter sp	CEP0684	-	-	-	+

Appendix 3.8 (cont'd..): Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc IV	CEP0194	++	-	-	+
Bcc III-B	CEP0499	+/-	-	-	+
Bcc III-A	CEP0715	+/-	-	-	+
Bcc III-A	CEP0749	++	-	-	+
Bcc II	C7273	+	-	-	+
Bcc V	CEP0086	+/-	-	-	+
Bcc IV	CEP0176	+	-	+	+
Bcc III-A	C4872	+++	-	-	+
Bcc III-A	CEP0236	++	-	-	+
Bcc V	CEP0480	+/-	-	-	+
Bcc III-A	CEP0787	+/-	-	-	+
Bcc I	CEP0957	+	-	+	+
Bcc IV	CEP1025	+	-	-	+
Bcc I	FC0449	+	-	-	+
Bcc V	FC0464	+	-	-	+
Bcc V	CEP0213	+/-	-	-	+
Bcc IV	CEP0500	+	-	+/-	+
Bcc III-A	CEP0168	+	-	-	+
Bcc IV	CEP0711	+	-	+/-	+
Bcc VI	CEP0735	-	-	-	+
Bcc I	CEP0069	++	+/-	-	+
Bcc II	C9346	+/-	-	-	+
Bcc IV	CEP0952	+	-	-	+
Bcc IV	CEP1075	+	-	-	+
Bcc II	CEP0503	+	-	-	+
Bcc III-A	CEP0211	+	+/-	-	+
Bcc II	CEP0493	+	-	-	+
Bcc V	D0439	+/-	-	-	+
Bcc III-A	CEP0209	+	+/-	-	+
Bcc I	CEP0843	+	-	-	+
Bcc II	C7363	+	-	-	+
Bcc III-A	C7837	+/-	-	-	+
Bcc II	C8982	+/-	-	-	+
Bcc I	CEP0067	+/-	-	-	+
Bcc V	CEP0741	+/-	-	-	+
Bcc III-B	C7946	+	+/-	-	+
Bcc II	CEP1018	+/-	-	-	+
Bcc VI	CEP1007	-	-	-	+
Bcc VI	CEP1014	+/-	-	-	+
Bcc VI	CEP0766	+/-	-	-	+
R. pickettii	CEP0096	-	-	-	++
A. denitrifications	CEP0184	-	-	-	+
A. denitrifications	CEP0183	-	-	-	+
A. faecalis	CEP0864	-	-	-	+
A. denitrifications	C9774	-	-	-	+
S. maltophilia	CEP0406	+/-	+++	+/-	+
S. maltophilia	CEP0272	+	+++	+/-	+
P. apista	C8757	-	-	-	+
P. pnomemusa	C7351	-	-	-	+

Appendix 3.8 (cont`d..): Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc VI	CEP1010	+/-	-	-	+
Bcc VI	CEP1008	+/-	-	-	+
Bcc V	CEP0649	-	-	-	+
R. pickettii	C6529	-	-	-	++
S. maltophilia	FC0827	+	+++	-	+
Bcc I	CEP1030	+	-	-	+
P. pnomenusa	C8029	-	-	-	+
R. pickettii	C8770	-	-	-	++
Bcc VIII	FC0972	+	-	-	+
Bcc VIII	FC0970	+	-	-	+
Bcc III-B	CEP0616	-	-	-	+
Bcc VIII	FC0973	+	-	-	+
Bcc VI	FC0346	-	-	-	+
Bcc VII	FC0662	+/-	-	-	+
Bcc V	CEP0639	-	-	-	+
Bcc I	CEP1032	+	-	-	+
A. xylosoxidans	D0423	-	-	-	+
Bcc III-A	CEP0498	++	-	-	+
Bcc III-A	CEP0655	+/-	-	-	+
A. xylosoxidans	FC0815	-	-	-	+
Bcc IV	C7838	+	-	-	+
Bcc I	C8729	+/-	-	-	+
Bcc III-A	CEP0880	+/-	-	-	+
Bcc I	CEP0101	+	-	+	+
Bcc III-A	CEP0785	+	-	-	+
Bcc V	CEP0041	-	-	-	+
Bcc II	C5394	+/-	-	-	+
Bcc IV	CEP0185	+	-	+	+
Bcc III-A	CEP1199	-	-	-	+
Bcc II	C5449	+	-	-	+
Bcc V	CEP0196	+	-	-	+
Bcc III-A	CEP0186	+	-	-	+
Bcc IV	CEP1081	+	-	-	+
Bcc I	CEP0070	+	-	-	+
Bcc I	CEP0072	+/-	-	+/-	+
Bcc III-B	CEP0117	+	-	+/-	+
Bcc I	CEP0972	+/-	-	-	+
R. pickettii	C1139	-	-	+	++
Bcc III-B	CEP1111	+	-	-	+
A. xylosoxidans	C2302	-	-	-	+
Bcc II	CEP0965	+	-	-	+
Bcc III-A	CEP0565	+	-	-	+
Bcc V	CEP0087	+/-	-	-	+
Bcc III-A	CEP1091	+/-	-	+	+
Bcc IV	CEP0118	+	-	-	+
Bcc V	C8766	-	-	-	+
Bcc III-B	CEP1113	+/-	-	-	+
Bcc VI	CEP0026	+/-	-	-	+
Bcc II	CEP0485	+/-	-	-	+

Appendix 3.8 (cont'd..): Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc I	CEP0081	+	-	-	+
Bcc I	CEP0068	+	-	+/-	+
Bcc III-B	CEP0114	+	-	-	+
Bcc IV	CEP0929	+	-	+/-	+
Bcc III-B	CEP0139	+	+/-	-	+
Bcc II	C9172	+	-	-	+
Bcc III-A	C6749	++	-	-	+
Bcc II	CEP1116	-	-	-	+
Bcc I	CEP0934	+	-	-	+
Bcc III-A	CEP0869	+	-	-	+
Bcc III-A	CEP1079	+	-	-	+
A. xylosoxidans	CEP1094	-	-	-	+
Bcc III-A	CEP0951	+	-	-	+
Bcc IV	C6061	+	-	-	+
Bcc II	CEP0786	+	-	-	+
Bcc IV	CEP0945	+	-	+/-	+
Bcc III-A	CEP0408	+	-	-	+
Bcc I	CEP1190	+	-	+/-	+
Bcc IX	FC0433	+	-	-	+
Bcc VI	CEP1012	-	-	-	+
Bcc III-B	1114	+/-	-	-	+
Bcc II	CEP1235	+	+/-	-	+
Bcc III-A	CEP0591	+	-	-	+
Bcc IV	FC0777	+/-	-	-	+
Bcc VIII	FC0969	+	-	-	+
A. xylosoxidans	FC0349	-	-	-	+
A. denitrifications	CEP0092	-	-	-	+
Bcc III-B	CEP0769	+	-	-	+
Bcc II	CEP0686	+/-	-	-	+
Bcc III-B	FC0378	+/-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
E. coli		-	+/-	-	+
E. cloacae		-	+/-	-	+
Bcc V	FC0465	+	-	-	+
Bcc I	CEP0615	+	+/-	-	+
Bcc II	FC0357	++	-	-	+
Bcc VI	FC0353	++	-	-	+
Bcc II	FC0102	+	-	-	+
Bcc II	CEP0691	++	-	-	+
Bcc III-A	CEP1161	+/-	-	-	+
Bcc III-A	FC0120	++	+/-	-	+
Bcc II	D0156	++	+/-	-	+
Bcc II	D0297	+	-	-	+
Bcc VI	CEP1013	+	-	-	+
Bcc IV	FC0362	++	-	-	+

Appendix 3.8 (cont'd..): Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc VIII	FC0961	++	-	-	+
Bcc VIII	FC0964	++	-	-	+
Bcc VIII	FC0965	+	-	-	+
Bcc III-B	CEP0628	++	+/-	+/-	+
Bcc III-A	CEP0768	+	-	-	+
Bcc II	CEP0699	++	+	-	+
Bcc III-B	CEP0986	+	-	-	+
Bcc II	CEP0455	+	-	-	+
Bcc V	C9710	++	-	-	+
Bcc III-A	CEP0215	++	+	-	+
Bcc II	C7637	++	-	-	+
Bcc V	C9287	++	-	-	+
Bcc III-B	CEP0187	++	-	-	+
Bcc IV	CEP0103	++	+/-	-	+
Bcc II	C8681	++	-	-	+
Bcc V	C9371	++	-	-	+
Bcc IV	CEP0235	++	-	-	+
Bcc I	CEP0240	+/-	+/-	-	+
Bcc VII	CEP0102	+	-	-	+
Bcc II	C5275	++	-	-	+
Bcc I	CEP0073	++	-	-	+
Bcc V	CEP0084	++	-	-	+
Bcc VII	CEP0617	++	-	-	+
Bcc II	CEP0169	++	-	-	+
Bcc V	C8952	++	-	-	+
Bcc III-A	CEP0146	++	-	-	+
Bcc I	CEP0076	+	-	+/-	+
Bcc V	CEP0192	+	-	-	+
Bcc III-A	CEP0606	+	-	+/-	+
Bcc V	CEP0047	+	-	-	+
R. pickettii	CEP0488	-	-	-	+++
Bcc III-A	CEP0931	+	-	-	+
Bcc IV	CEP0404	+	-	+/-	+
Bcc II	C7263	+	-	-	+
Bcc I	C8536	+	-	-	+
Bcc V	CEP0233	++	-	-	+
Bcc IV	CEP0142	+	-	-	+
Bcc II	D0056	+	+/-	-	+
Bcc I	CEP0074	+	-	-	+
Bcc II	CEP0243	+	-	-	+
Bcc II	D0155	+	-	-	+
Bcc V	FC0373	+/-	-	-	+
Bcc I	CEP1140	+	+/-	+/-	+
Bcc V	D0278	+	++	-	+
Bcc II	C7329	+	-	-	+
Bcc V	FC0622	+	-	-	+
Bcc III-A	CEP001	+	-	-	+
Bcc V	CEP1236	+	-	-	+
Bcc II	CEP0601	+	-	-	+

Appendix 3.8 (cont'd..): Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc II	CEP0108	+	-	-	+
Bcc V	CEP0175	+	+/-	-	+
Bcc I	CEP0990	+	-	+/-	+
Bcc II	CEP1000	+	-	-	+
Bcc V	CEP0865	+	-	-	+
Bcc IV	CEP1064	+	-	-	+
Bcc VII	CEP0958	+	-	-	+
Bcc I	CEP0834	+	-	-	+
Bcc III-B	CEP0054	+	-	-	+
Bcc III-A	CEP0156	+	-	-	+
Bcc IV	CEP0846	+	++	-	+
Bcc IV	CEP0851	++	-	-	+
A. xylosoxidans	C3968	-	-	-	+
Bcc V	CEP0982	+/-	-	-	+
Bcc VI	CEP0873	+	-	-	+
Bcc IV	FC0473	++	-	-	+
Bcc VI	CEP0021	+	+/-	-	+
Bcc IV	CEP0710	++	-	-	+
Bcc III-B	CEP1067	++	+/-	+/-	+
Bcc VII	CEP0996	+	-	-	+
Bcc VII	CEP1232	+	-	+/-	+
Bcc I	FC0460	+	+	-	+
Bcc VI	CEP0743	+/-	-	-	+
Bcc I	FC0660	+	+/-	+/-	+
Bcc VI	CEP1011	+	-	-	+
Bcc II	CEP1129	+	-	-	+
Bcc III-A	FC1057	+	+	+	+
Bcc I	FC0457	+	+/-	-	+
Bcc I	CEP1132	+	-	-	+
Bcc V	CEP1224	+	-	-	+
Bcc V	SQ004C	+	-	-	+
Bcc II	CEP0630	+	-	-	+
Bcc VIII	FC0974	++	-	-	+
Bcc III-B	CEP1119	++	+	+/-	+
Bcc III-A	CEP0703	+/-	-	-	+
Bcc V	CEP0706	+/-	-	-	+
Bcc VII	FC0881	+	+	+++	+
Bcc IV	FC0772	+	-	-	+
Bcc II	CEP1129	+	-	-	+
Bcc V	D0121	+/-	+	-	+
Bcc IV	CEP0059	+	-	-	+
Bcc V	CEP0255	+	-	-	+
Bcc I	FC1104	+	-	-	+
Bcc VIII	FC0962	+	-	-	+
Bcc VI	FC0380	+	+/-	-	+
Bcc VIII	FC0963	+	-	-	+
Bcc II	D0445	+/-	-	-	+
Bcc III-A	CEP0702	+/-	-	-	+
Bcc III-A	CEP0300	+	-	-	+

Appendix 3.8 (cont'd..): Increases in absorbance (405 nm) caused by various bacteria from Collection B due to hydrolysis of chromogenic substrates after 48 hr incubation

		Substrate			
Strain	**Reference**	*p* NP phe-phos	H-glu-gly-arg-*p* NA	Suc-phe-leu-phe-*p* NA	*p* NP-guano
Bcc III-B	FC0372	+	+	-	+
Bcc II	FC0898	+/-	-	-	+
Bcc VIII	FC0967	+	-	-	+
Bcc III-A	FC0506	+	+/-	-	+
Bcc VII	FC0623	+	-	-	+
Bcc VII	FC0882	+/-	-	-	+
Bcc III-B	FC0802	+/-	++	-	+
Bcc IV	FC0503	+/-	-	+	+
Bcc III-B	FC0499	+/-	-	-	+
Bcc IX	FC0451	+/-	-	-	+
Bcc V	FC0463	+	-	-	+
Bcc III-A	CEP0635	+	+/-	-	+
Bcc III-B	CEP1184	-	-	-	+
Bcc I	FC1107	+	-	-	+
Bcc IV	FC0778	+	-	-	+
Bcc VIII	FC0968	+	-	-	+
Bcc III-B	CEP1139	+	+/-	+/-	+
Bcc VIII	FC0976	+/-	-	-	+
Bcc I	FC1108	+/-	-	-	+
Bcc I	CEP1151	+	-	-	+
Bcc VII	FC0767	+	-	-	+
Bcc I	FC0649	+	-	-	+
Bcc V	FC0659	+	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
H_2O		-	-	-	+
E. coli		-	-	-	+
E. cloacae		-	+/-	+/-	+

Key:

Bcc I, *B. cepacia*; Bcc II, *B. multivorans*; Bcc III, *B. cenocepacia*; Bcc IV, *B. stabilis*; Bcc V, *B. vietnamiensis*; Bcc VI, *B. dolosa*; Bcc VII, *B. ambifaria*; Bcc VIII, *B. anthina*; Bcc IX, *B. pyrrocinia*

p NP-phe-phos; *para*-nitrophenyl-phenyl-phosphonate; H-glu-gly-arg-*p* NA, H-glutamate-glycyl-arginyl-para-nitroanilide; Suc-phe-leu-phe-*p* NA, Succinyl-phenylalanyl-leucyl-phenylalanyl-*para*-nitroanilide; *p* NP-p-guano, *para*-nitrophenyl-guanidinobenzoate

Appendix 3.9: Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate						
Strain	**Reference**	Control	Glycerol	Erythritol	D-Arabinose	L-Arabinose	Ribose	D-Xylose
B. cepacia	LMG 1222	0	1	0	4	5	2	3
B. cepacia	LMG 2161	0	0	0	1	2	2	0
B. cenocepacia	LMG 16654	0	0	0	1	3	0	2
B. cenocepacia	LMG 16656	0	0	0	1	2	0	1
B. cenocepacia	LMG 16659	0	0	0	4	5	0	2
B. cepacia	LMG 17997	0	0	0	1	2	3	1
B. cepacia	LMG 18821	0	0	0	0	1	0	2
B. cenocepacia	LMG 18826	0	5	0	0	1	0	4
B. cenocepacia	LMG 18827	0	5	0	2	5	2	5
B. cenocepacia	LMG 18828	0	4	0	3	4	3	5
B. cenocepacia	LMG 18829	0	0	0	1	2	0	1
B. cenocepacia	LMG 18830	0	5	0	3	4	2	4
B. cenocepacia	LMG 18832	0	0	0	4	4	0	4
B. cenocepacia	LMG 18863	0	0	0	2	2	0	1
B. multivorans	LMG 13010	0	5	0	1	3	0	5
B. multivorans	LMG 16660	0	5	0	1	3	1	5
B. multivorans	LMG 16665	0	0	0	1	1	0	1
B. multivorans	LMG 17588	0	1	0	2	5	0	5
B. multivorans	LMG 18822	0	1	0	1	5	0	5
B. multivorans	LMG 18823	0	1	0	2	5	1	5
B. multivorans	LMG 18824	0	1	0	3	5	1	4
B. multivorans	LMG 18825	0	3	0	3	5	1	5
B. stabilis	LMG 14086	0	0	0	3	1	0	0
B. stabilis	LMG 14294	0	1	0	1	5	0	5
B. stabilis	LMG 18870	0	0	0	1	5	0	3
B. stabilis	LMG 18888	0	5	0	5	2	0	2
B. vietnamiensis	LMG 10929	0	1	0	1	1	0	1
B. vietnamiensis	LMG 16232	0	0	0	1	3	0	2
B. vietnamiensis	LMG 18835	0	0	0	0	2	0	2
B. vietnamiensis	LMG 18836	0	5	0	4	5	1	5

Appendix 3.9 (cont`d..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate					
Strain	**Reference**	L-Xylose	Adonitol	ß-Methyl-xyloside	Galactose	D-Glucose	D-Fructose
B. cepacia	LMG 1222	0	3	0	5	4	2
B. cepacia	LMG 2161	0	3	0	4	4	3
B. cenocepacia	LMG 16654	0	1	0	5	5	2
B. cenocepacia	LMG 16656	0	1	0	5	1	0
B. cenocepacia	LMG 16659	0	5	0	5	5	2
B. cepacia	LMG 17997	0	1	0	5	5	1
B. cepacia	LMG 18821	0	3	0	5	5	2
B. cenocepacia	LMG 18826	0	0	0	5	4	0
B. cenocepacia	LMG 18827	0	1	0	5	3	5
B. cenocepacia	LMG 18828	1	0	0	5	4	1
B. cenocepacia	LMG 18829	0	1	0	5	5	0
B. cenocepacia	LMG 18830	0	0	0	5	4	4
B. cenocepacia	LMG 18832	1	4	0	5	5	5
B. cenocepacia	LMG 18863	0	1	0	5	5	5
B. multivorans	LMG 13010	0	2	0	5	5	2
B. multivorans	LMG 16660	0	0	0	5	4	2
B. multivorans	LMG 16665	0	1	0	5	5	1
B. multivorans	LMG 17588	0	5	0	5	5	5
B. multivorans	LMG 18822	0	5	1	5	5	5
B. multivorans	LMG 18823	0	5	0	5	5	5
B. multivorans	LMG 18824	1	4	1	5	5	5
B. multivorans	LMG 18825	1	5	1	5	5	5
B. stabilis	LMG 14086	0	0	0	5	5	3
B. stabilis	LMG 14294	0	5	1	5	5	5
B. stabilis	LMG 18870	0	1	0	5	5	4
B. stabilis	LMG 18888	2	5	2	5	5	5
B. vietnamiensis	LMG 10929	0	0	0	5	5	4
B. vietnamiensis	LMG 16232	0	1	0	5	5	3
B. vietnamiensis	LMG 18835	0	1	0	5	5	2
B. vietnamiensis	LMG 18836	0	0	0	5	5	5

Appendix 3.9 (cont'd..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate					
Strain	**Reference**	Rhamnose	Dulcitol	Inositol	Mannitol	Sorbitol	α-Methyl-D-mannoside
B. cepacia	LMG 1222	0	2	5	4	4	0
B. cepacia	LMG 2161	0	3	5	5	5	0
B. cenocepacia	LMG 16654	0	1	4	3	4	0
B. cenocepacia	LMG 16656	0	0	3	2	2	0
B. cenocepacia	LMG 16659	0	2	5	5	5	0
B. cepacia	LMG 17997	0	1	3	4	3	0
B. cepacia	LMG 18821	5	4	5	5	5	0
B. cenocepacia	LMG 18826	0	0	0	3	0	0
B. cenocepacia	LMG 18827	0	1	5	4	3	0
B. cenocepacia	LMG 18828	0	0	0	2	0	0
B. cenocepacia	LMG 18829	0	0	3	1	1	0
B. cenocepacia	LMG 18830	0	0	5	5	1	0
B. cenocepacia	LMG 18832	0	4	5	5	5	0
B. cenocepacia	LMG 18863	0	2	5	5	5	0
B. multivorans	LMG 13010	0	0	0	0	0	0
B. multivorans	LMG 16660	0	0	0	5	0	0
B. multivorans	LMG 16665	0	0	2	2	2	0
B. multivorans	LMG 17588	0	5	5	5	5	0
B. multivorans	LMG 18822	0	5	5	5	5	0
B. multivorans	LMG 18823	0	5	5	5	5	0
B. multivorans	LMG 18824	0	4	5	5	5	0
B. multivorans	LMG 18825	0	5	5	5	5	0
B. stabilis	LMG 14086	0	0	1	0	0	0
B. stabilis	LMG 14294	0	5	5	5	5	0
B. stabilis	LMG 18870	0	3	4	4	3	0
B. stabilis	LMG 18888	0	5	5	5	5	0
B. vietnamiensis	LMG 10929	0	0	5	4	4	0
B. vietnamiensis	LMG 16232	0	0	1	1	1	0
B. vietnamiensis	LMG 18835	0	1	1	1	1	0
B. vietnamiensis	LMG 18836	0	5	5	5	5	0

Appendix 3.9 (cont`d..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate				
Strain	**Reference**	α-Methyl-D-glucoside	N acetyl glucosamide	Amygdaline	Arbutine	Esculine
B. cepacia	LMG 1222	0	0	1	5	0
B. cepacia	LMG 2161	0	0	5	3	0
B. cenocepacia	LMG 16654	0	0	0	2	5
B. cenocepacia	LMG 16656	0	0	0	4	5
B. cenocepacia	LMG 16659	0	0	1	5	5
B. cepacia	LMG 17997	0	0	1	4	5
B. cepacia	LMG 18821	0	0	0	4	5
B. cenocepacia	LMG 18826	0	0	0	0	0
B. cenocepacia	LMG 18827	0	0	0	0	0
B. cenocepacia	LMG 18828	0	0	0	0	2
B. cenocepacia	LMG 18829	0	0	0	3	5
B. cenocepacia	LMG 18830	0	0	0	4	5
B. cenocepacia	LMG 18832	0	0	0	5	5
B. cenocepacia	LMG 18863	0	0	0	5	5
B. multivorans	LMG 13010	0	0	0	0	0
B. multivorans	LMG 16660	0	0	0	0	0
B. multivorans	LMG 16665	0	0	0	2	5
B. multivorans	LMG 17588	0	0	0	0	0
B. multivorans	LMG 18822	0	0	0	0	0
B. multivorans	LMG 18823	0	0	0	0	0
B. multivorans	LMG 18824	0	0	0	0	0
B. multivorans	LMG 18825	0	0	0	0	0
B. stabilis	LMG 14086	0	0	1	1	5
B. stabilis	LMG 14294	0	0	0	0	0
B. stabilis	LMG 18870	0	0	0	0	5
B. stabilis	LMG 18888	0	0	0	1	0
B. vietnamiensis	LMG 10929	0	0	0	0	0
B. vietnamiensis	LMG 16232	0	0	0	0	0
B. vietnamiensis	LMG 18835	0	0	0	0	0
B. vietnamiensis	LMG 18836	0	0	0	0	0

Appendix 3.9 (cont`d..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

Strain	Reference	Salicine	Cellobiose	Maltose	Lactose	Melibiose	Saccharose	Trehalose	Inuline
B. cepacia	LMG 1222	2	2	2	5	0	2	1	0
B. cepacia	LMG 2161	5	2	1	3	1	4	0	0
B. cenocepacia	LMG 16654	1	1	1	1	0	1	0	0
B. cenocepacia	LMG 16656	2	2	2	3	0	2	1	0
B. cenocepacia	LMG 16659	5	4	3	4	2	5	0	0
B. cepacia	LMG 17997	4	3	5	5	0	4	2	0
B. cepacia	LMG 18821	2	1	2	3	0	4	0	0
B. cenocepacia	LMG 18826	0	0	0	0	0	4	0	0
B. cenocepacia	LMG 18827	0	0	0	5	0	0	0	0
B. cenocepacia	LMG 18828	0	0	0	0	0	0	0	0
B. cenocepacia	LMG 18829	2	2	2	3	0	2	1	0
B. cenocepacia	LMG 18830	0	0	3	2	0	5	1	0
B. cenocepacia	LMG 18832	5	3	2	3	0	5	1	0
B. cenocepacia	LMG 18863	5	2	5	3	0	5	3	0
B. multivorans	LMG 13010	0	0	0	0	0	0	0	0
B. multivorans	LMG 16660	0	0	0	0	0	0	0	0
B. multivorans	LMG 16665	1	1	1	1	0	2	1	0
B. multivorans	LMG 17588	0	4	5	4	0	3	5	0
B. multivorans	LMG 18822	0	2	1	2	0	0	5	0
B. multivorans	LMG 18823	0	4	3	5	0	0	5	0
B. multivorans	LMG 18824	0	4	5	4	0	4	5	0
B. multivorans	LMG 18825	0	2	2	3	0	0	5	0
B. stabilis	LMG 14086	0	3	4	3	0	4	5	0
B. stabilis	LMG 14294	0	3	5	5	0	3	5	0
B. stabilis	LMG 18870	0	4	3	5	0	1	5	0
B. stabilis	LMG 18888	0	5	5	5	5	0	5	0
B. vietnamiensis	LMG 10929	0	1	0	2	0	3	0	0
B. vietnamiensis	LMG 16232	0	2	4	5	0	1	5	0
B. vietnamiensis	LMG 18835	0	2	2	4	0	0	5	0
B. vietnamiensis	LMG 18836	0	0	0	3	0	5	0	0

Appendix 3.9 (cont`d..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate						
Strain	**Reference**	Melezitose	D-Raffinose	Amidon	Glycogene	Xylitol	ß-Gentiobiose	D-Turanose
B. cepacia	LMG 1222	0	0	0	0	0	0	0
B. cepacia	LMG 2161	0	1	0	0	0	0	0
B. cenocepacia	LMG 16654	0	0	0	0	0	0	0
B. cenocepacia	LMG 16656	0	0	0	0	0	0	0
B. cenocepacia	LMG 16659	0	1	0	0	0	0	0
B. cepacia	LMG 17997	0	4	0	0	0	0	0
B. cepacia	LMG 18821	0	0	0	0	0	0	0
B. cenocepacia	LMG 18826	0	0	0	0	0	0	0
B. cenocepacia	LMG 18827	0	0	0	0	0	0	0
B. cenocepacia	LMG 18828	0	0	0	0	0	0	0
B. cenocepacia	LMG 18829	0	0	0	0	0	0	0
B. cenocepacia	LMG 18830	0	0	0	0	0	0	0
B. cenocepacia	LMG 18832	0	0	0	0	0	0	0
B. cenocepacia	LMG 18863	0	0	0	0	0	0	0
B. multivorans	LMG 13010	0	0	0	0	0	0	0
B. multivorans	LMG 16660	0	0	0	0	0	0	0
B. multivorans	LMG 16665	0	0	0	0	0	0	0
B. multivorans	LMG 17588	0	0	0	0	0	0	0
B. multivorans	LMG 18822	0	0	0	0	0	0	0
B. multivorans	LMG 18823	0	0	0	0	0	0	0
B. multivorans	LMG 18824	0	0	0	0	0	0	0
B. multivorans	LMG 18825	0	0	0	0	0	0	0
B. stabilis	LMG 14086	0	0	0	0	0	3	0
B. stabilis	LMG 14294	0	0	0	0	0	0	0
B. stabilis	LMG 18870	0	0	0	0	0	0	0
B. stabilis	LMG 18888	0	5	0	0	5	5	0
B. vietnamiensis	LMG 10929	0	0	0	0	0	0	0
B. vietnamiensis	LMG 16232	0	0	0	0	0	0	0
B. vietnamiensis	LMG 18835	0	0	0	0	0	0	0
B. vietnamiensis	LMG 18836	0	0	0	0	0	0	0

Appendix 3.9 (cont'd..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate						
Strain	**Reference**	D-Lyxose	D-Tagatose	D-Fucose	L-Fucose	D-Arabitol	L-Arabitol	Gluconate
B. cepacia	LMG 1222	1	0	5	1	1	1	0
B. cepacia	LMG 2161	1	0	5	4	4	4	0
B. cenocepacia	LMG 16654	0	0	3	0	0	0	0
B. cenocepacia	LMG 16656	0	0	5	1	0	0	0
B. cenocepacia	LMG 16659	0	0	4	1	1	1	0
B. cepacia	LMG 17997	1	0	4	0	0	0	0
B. cepacia	LMG 18821	0	0	1	0	0	1	0
B. cenocepacia	LMG 18826	0	0	4	0	0	0	0
B. cenocepacia	LMG 18827	0	0	3	0	0	0	0
B. cenocepacia	LMG 18828	0	0	5	0	0	0	0
B. cenocepacia	LMG 18829	1	0	5	1	1	0	0
B. cenocepacia	LMG 18830	0	0	5	1	0	0	0
B. cenocepacia	LMG 18832	0	0	3	1	1	1	0
B. cenocepacia	LMG 18863	0	0	2	0	0	0	0
B. multivorans	LMG 13010	0	0	4	0	0	0	0
B. multivorans	LMG 16660	0	0	4	0	0	0	0
B. multivorans	LMG 16665	0	0	3	1	0	0	0
B. multivorans	LMG 17588	0	0	5	2	4	2	0
B. multivorans	LMG 18822	0	0	3	0	1	0	0
B. multivorans	LMG 18823	0	0	5	0	4	1	0
B. multivorans	LMG 18824	2	1	5	2	2	2	0
B. multivorans	LMG 18825	1	0	5	2	5	5	0
B. stabilis	LMG 14086	0	0	0	1	0	0	0
B. stabilis	LMG 14294	0	0	5	1	1	1	0
B. stabilis	LMG 18870	0	0	5	0	0	0	0
B. stabilis	LMG 18888	0	0	4	5	5	5	0
B. vietnamiensis	LMG 10929	0	0	1	0	0	0	0
B. vietnamiensis	LMG 16232	0	0	3	0	0	0	0
B. vietnamiensis	LMG 18835	0	0	3	0	0	0	0
B. vietnamiensis	LMG 18836	2	0	2	0	5	0	0

Appendix 3.9 (cont`d..): Scores of colour intensity recordings of carbohydrate oxidation reactions from 50CHstrips on 30 BCCM reference panel of Bcc strains after 48 hr incubation

		Carbohydrate substrate	
Strain	**Reference**	2 Keto-gluconate	5 Keto-gluconate
B. cepacia	LMG 1222	0	0
B. cepacia	LMG 2161	0	0
B. cenocepacia	LMG 16654	0	0
B. cenocepacia	LMG 16656	0	0
B. cenocepacia	LMG 16659	0	0
B. cepacia	LMG 17997	0	0
B. cepacia	LMG 18821	0	0
B. cenocepacia	LMG 18826	0	0
B. cenocepacia	LMG 18827	0	0
B. cenocepacia	LMG 18828	0	0
B. cenocepacia	LMG 18829	0	0
B. cenocepacia	LMG 18830	0	0
B. cenocepacia	LMG 18832	0	0
B. cenocepacia	LMG 18863	0	0
B. multivorans	LMG 13010	0	0
B. multivorans	LMG 16660	0	0
B. multivorans	LMG 16665	0	0
B. multivorans	LMG 17588	0	0
B. multivorans	LMG 18822	0	0
B. multivorans	LMG 18823	0	0
B. multivorans	LMG 18824	0	0
B. multivorans	LMG 18825	0	0
B. stabilis	LMG 14086	0	0
B. stabilis	LMG 14294	0	0
B. stabilis	LMG 18870	0	0
B. stabilis	LMG 18888	0	0
B. vietnamiensis	LMG 10929	0	0
B. vietnamiensis	LMG 16232	0	0
B. vietnamiensis	LMG 18835	0	0
B. vietnamiensis	LMG 18836	0	0

Appendix 3.10: Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0717	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0506	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc V	CEP0126	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0824	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0060	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0469	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0999	red	red	red	red	red	red	red	red	red	yellow	yellow	red
Bcc IIIb	CEP0762	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	C4708	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0562	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0112	red	red	red	red	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0519	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc VI	CEP0028	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc II	C8467	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc IIIa	CEP0198	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc II	C9281	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0444	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1006	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0533	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0507	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0773	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP1110	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0961	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc V	CEP0974	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0027	red	red	red	red	red	red	red	red	red	red	yellow	yellow
Bcc V	CEP0339	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0136	red	red	red	red	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP0942	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0143	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0505	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC0427	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0181	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc IIIb	CEP0750	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0663	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C4356	red	red	red	red	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	CEP0242	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc II	CEP0978	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc IIIa	C6279	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0516	red	red	red	red	red	red	red	red	red	red	yellow	yellow
Bcc IIIa	CEP0755	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1114	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0938	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0984	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc IV	CEP0194	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0499	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc IIIa	CEP0715	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IIIa	CEP0749	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7273	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc V	CEP0086	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc IV	CEP0176	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	C4872	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0236	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0480	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0787	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0957	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1025	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0449	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0464	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0213	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0500	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0168	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc IV	CEP0711	red	red	red	red	red	red	red	red	red	orange	orange	orange
Bcc VI	CEP0735	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0069	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C9346	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0952	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1075	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0503	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0211	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0493	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	D0439	red	red	red	red	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0209	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0843	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7363	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	C7837	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C8982	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0067	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0741	red	red	red	red	red	red	red	red	red	red	orange	orange
Bcc IIIb	C7946	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc II	CEP1018	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1007	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc VI	CEP1014	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0766	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1010	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1008	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc V	CEP0649	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1030	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0972	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0970	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0616	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0973	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc VI	FC0346	red	red	red	red	red	red	red	red	red	red	red	orange

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	FC0662	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc V	CEP0639	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc I	CEP1032	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0498	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0655	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	C7838	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	C8729	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0880	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0101	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0785	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0041	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc II	C5394	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0185	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP1199	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C5449	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc V	CEP0196	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0186	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1081	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0070	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0072	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0117	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0972	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1111	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc II	CEP0965	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0565	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0087	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP1091	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0118	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	C8766	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1113	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0026	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0485	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0081	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0068	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0114	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0929	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0139	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C9172	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	C6749	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1116	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0934	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0869	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP1079	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0951	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	C6061	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0786	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0945	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0408	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1190	red	red	red	red	red	red	red	red	red	red	red	red
BPY	FC0433	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1012	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	1114	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1235	red	red	red	red	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP0591	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0777	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0969	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0769	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0686	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0378	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0465	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0615	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0357	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	FC0353	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0102	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0691	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP1161	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC0120	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	D0156	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	D0297	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1013	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0362	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0961	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0964	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0965	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0628	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc IIIa	CEP0768	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0699	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0986	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0455	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	C9710	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0215	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7637	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	C9287	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0187	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0103	red	red	red	red	red	red	red	red	red	red	red	yellow
Bcc II	C8681	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	C9371	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0235	red	red	red	red	red	red	red	red	yellow	orange	orange	red
Bcc I	CEP0240	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0102	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C5275	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0073	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc V	CEP0084	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0617	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0169	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	C8952	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0146	red	red	red	red	red	red	red	red	red	orange	yellow	yellow
Bcc I	CEP0076	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0192	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0606	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0047	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0931	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0404	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7263	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	C8536	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0233	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0142	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	D0056	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0074	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0243	red	red	red	red	red	red	red	red	red	red	orange	yellow
Bcc II	D0155	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0373	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1140	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	D0278	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7329	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0622	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP001	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP1236	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0601	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0108	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0175	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0990	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1000	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0865	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1064	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0958	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0834	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0054	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0156	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0846	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0851	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0982	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0873	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0473	red	red	red	red	red	red	red	red	red	red	orange	orange
Bcc VI	CEP0021	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc IV	CEP0710	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1067	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0996	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	CEP1232	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0460	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0743	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0660	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1011	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1129	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC1057	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0457	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1132	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP1224	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	SQ004C	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0630	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0974	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1119	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0703	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0706	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0881	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc IV	FC0772	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1129	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	D0121	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0059	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0255	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC1104	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0962	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	FC0380	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0963	red	red	red	red	red	red	red	red	red	red	red	orange
Bcc II	D0445	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0702	red	red	red	red	red	red	red	red	red	orange	red	red
Bcc IIIa	CEP0300	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0372	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0898	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0967	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC0506	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0623	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0882	red	red	red	red	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIb	FC0802	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0503	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0499	red	red	red	red	red	red	red	red	red	red	red	red
BPY	FC0451	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0463	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0635	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1184	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC1107	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0778	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0968	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1139	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Substrate free control 1				Adonitol				D-Fructose			
Strain	**Reference**	**48**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VIII	FC0976	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC1108	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1151	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0767	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0649	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0659	red	red	red	red	red	red	red	red	red	red	red	red
Positive control	see below	red	red	red	red	red	red	red	red	red	red	red	yellow
Org free control		red	red	red	red	red	red	red	red	red	red	red	red
Org free control		red	red	red	red	red	red	red	red	red	red	red	red

Positive control Bcc strains

Carbohydrate	**Reference**
Adonitol	LMG 16659
D-fructose	LMG 18827

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0717	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0506	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0126	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IIIa	CEP0824	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0060	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0469	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc V	CEP0999	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0762	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	C4708	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0562	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0112	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIb	CEP0519	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP0028	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	C8467	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0198	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	C9281	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0444	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc II	CEP1006	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0533	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0507	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0773	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc V	CEP1110	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIb	CEP0961	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0974	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc VI	CEP0027	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0339	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0136	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0942	red	yellow	yellow	orange	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0143	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc V	CEP0505	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IIIa	FC0427	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc II	CEP0181	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0750	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0663	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc II	C4356	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0242	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0978	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	C6279	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc VII	CEP0516	red	red	red	red	yellow	yellow	orange	red	red	red	red	red
Bcc IIIa	CEP0755	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1114	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0938	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0984	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0194	red	orange	yellow	orange	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0499	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0715	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IIIa	CEP0749	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc II	C7273	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0086	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc IV	CEP0176	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	C4872	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0236	red	red	orange	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0480	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0787	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0957	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc IV	CEP1025	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc I	FC0449	red	red	red	red	red	orange	orange	yellow	red	red	red	red
Bcc V	FC0464	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc V	CEP0213	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0500	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0168	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0711	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP0735	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0069	red	red	orange	orange	red	red	red	yellow	red	red	red	red
Bcc II	C9346	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IV	CEP0952	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IV	CEP1075	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc II	CEP0503	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0211	red	red	red	red	yellow	yellow	yellow	orange	red	red	red	red
Bcc II	CEP0493	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	D0439	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0209	red	red	red	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0843	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc II	C7363	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	C7837	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc II	C8982	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0067	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0741	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	C7946	red	red	red	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP1018	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP1007	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP1014	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP0766	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc VI	CEP1010	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP1008	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0649	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc I	CEP1030	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc VIII	FC0972	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VIII	FC0970	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0616	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc VIII	FC0973	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc VI	FC0346	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	FC0662	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0639	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP1032	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0498	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0655	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	C7838	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	C8729	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IIIa	CEP0880	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0101	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0785	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0041	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc II	C5394	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0185	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP1199	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	C5449	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0196	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0186	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP1081	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0070	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0072	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIb	CEP0117	red	red	orange	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0972	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	CEP1111	red	red	red	red	yellow	yellow	yellow	o	red	red	red	red
Bcc II	CEP0965	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0565	red	red	yellow	yellow	yellow	yellow	yellow	red	red	red	red	red
Bcc V	CEP0087	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IIIa	CEP1091	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0118	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	C8766	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	CEP1113	red	red	red	red	red	red	red	o	red	red	red	red
Bcc VI	CEP0026	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0485	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0081	red	red	orange	yellow	red	red	orange	yellow	red	red	red	red
Bcc I	CEP0068	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0114	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0929	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0139	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	C9172	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	C6749	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP1116	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc I	CEP0934	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0869	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc IIIa	CEP1079	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0951	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IV	C6061	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0786	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0945	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0408	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1190	red	red	red	red	red	red	red	o	red	red	red	red
BPY	FC0433	red	red	red	red	red	red	red	o	red	red	red	red
Bcc VI	CEP1012	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	1114	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP1235	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0591	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0777	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0969	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0769	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0686	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	FC0378	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc V	FC0465	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc I	CEP0615	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0357	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	FC0353	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	FC0102	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0691	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP1161	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC0120	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	D0156	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	D0297	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP1013	red	red	red	red	red	red	red	o	red	red	red	red
Bcc IV	FC0362	red	red	red	red	red	red	red	o	red	red	red	red
Bcc VIII	FC0961	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc VIII	FC0964	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VIII	FC0965	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0628	red	red	red	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0768	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0699	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0986	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0455	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	C9710	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0215	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7637	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc V	C9287	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0187	red	red	red	red	red	red	yellow	red	red	red	red	red
Bcc IV	CEP0103	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C8681	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc V	C9371	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IV	CEP0235	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0240	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0102	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	C5275	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0073	red	red	red	red	red	orange	yellow	yellow	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc V	CEP0084	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0617	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0169	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	C8952	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0146	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0076	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc V	CEP0192	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc IIIa	CEP0606	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0047	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0931	red	red	red	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0404	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7263	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc I	C8536	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc V	CEP0233	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IV	CEP0142	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc II	D0056	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0074	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc II	CEP0243	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	D0155	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	FC0373	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc I	CEP1140	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc V	D0278	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7329	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	FC0622	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc IIIa	CEP001	red	red	red	red	yellow	orange	orange	yellow	red	red	red	red
Bcc V	CEP1236	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0601	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0108	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0175	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0990	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc II	CEP1000	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0865	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1064	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0958	red	red	red	red	yellow	yellow	red	red	red	red	red	red
Bcc I	CEP0834	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0054	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0156	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0846	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0851	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc V	CEP0982	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc VI	CEP0873	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	FC0473	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc VI	CEP0021	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0710	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIb	CEP1067	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc VII	CEP0996	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	CEP1232	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc I	FC0460	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc VI	CEP0743	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	FC0660	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc VI	CEP1011	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP1129	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	FC1057	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0457	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc I	CEP1132	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP1224	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc V	SQ004C	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc II	CEP0630	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc VIII	FC0974	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP1119	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0703	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc V	CEP0706	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0881	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IV	FC0772	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1129	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc V	D0121	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0059	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0255	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	FC1104	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc VIII	FC0962	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	FC0380	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VIII	FC0963	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	D0445	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0702	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0300	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0372	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0898	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VIII	FC0967	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	FC0506	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0623	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc VII	FC0882	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	FC0802	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	FC0503	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0499	red	red	red	red	red	red	yellow	yellow	red	red	red	red
BPY	FC0451	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0463	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0635	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1184	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC1107	red	red	red	red	red	red	orange	yellow	red	red	red	red
Bcc IV	FC0778	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc VIII	FC0968	red	red	red	red	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP1139	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Salicine				Cellobiose				Substrate free control 2			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VIII	FC0976	red	red	red	red	orange	yellow	yellow	yellow	red	red	red	red
Bcc I	FC1108	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc I	CEP1151	red	red	red	red	red	orange	yellow	yellow	red	red	red	red
Bcc VII	FC0767	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	FC0649	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0659	red	red	red	red	red	red	orange	yellow	red	red	red	red
Positive control	see below	red	red	red	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Org free control		red	red	red	red	red	red	red	red	red	red	red	red
Org free control		red	red	red	red	red	red	red	red	red	red	red	red

Positive control Bcc strains

Carbohydrate	**Reference**
Salicin	LMG 16659
Cellobiose	LMG 18888

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0717	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0506	red	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc V	CEP0126	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0824	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0060	red	orange	orange	orange	red	red	red	red	red	red	red	red
Bcc IV	CEP0469	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0999	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0762	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc IIIa	C4708	red	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0562	red	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc IV	CEP0112	red	orange	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc IIIb	CEP0519	yellow	yellow	yellow	orange	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP0028	yellow	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	C8467	red	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0198	orange	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc II	C9281	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0444	red	red	red	orange	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP1006	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0533	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0507	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0773	red	red	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc V	CEP1110	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0961	red	orange	orange	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc V	CEP0974	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0027	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0339	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0136	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0942	yellow	yellow	orange	orange	red	orange	yellow	yellow	red	red	red	red
Bcc V	CEP0143	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0505	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC0427	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0181	yellow	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0750	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0663	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc II	C4356	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0242	red	orange	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0978	orange	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc IIIa	C6279	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0516	red	red	orange	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0755	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1114	red	orange	yellow	yellow	orange	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0938	red	yellow	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc IIIb	CEP0984	yellow	yellow	orange	orange	red	red	yellow	yellow	red	red	red	red
Bcc IV	CEP0194	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0499	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0715	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IIIa	CEP0749	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7273	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0086	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc IV	CEP0176	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	C4872	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0236	red	yellow	yellow	yellow	red	red	red	orange	red	red	red	red
Bcc V	CEP0480	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0787	red	orange	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0957	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1025	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0449	red	orange	orange	orange	red	red	red	red	red	red	red	red
Bcc V	FC0464	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0213	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0500	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0168	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0711	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc VI	CEP0735	red	red	yellow	yellow	red	red	red	red	red	red	red	red
Bcc I	CEP0069	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc II	C9346	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc IV	CEP0952	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc IV	CEP1075	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0503	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0211	yellow	yellow	yellow	orange	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0493	red	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc V	D0439	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0209	yellow	yellow	yellow	orange	red	orange	yellow	yellow	red	red	red	red
Bcc I	CEP0843	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	C7363	yellow	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	C7837	red	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	C8982	red	orange	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc I	CEP0067	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0741	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	C7946	orange	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc II	CEP1018	red	orange	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VI	CEP1007	red	red	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VI	CEP1014	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc VI	CEP0766	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc VI	CEP1010	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VI	CEP1008	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0649	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1030	red	yellow	yellow	yellow	red	red	orange	red	red	red	red	red
Bcc VIII	FC0972	red	yellow	yellow	yellow	red	red	orange	red	red	red	red	red
Bcc VIII	FC0970	red	yellow	yellow	yellow	red	red	orange	red	red	red	red	red
Bcc IIIb	CEP0616	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0973	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VI	FC0346	yellow	yellow	yellow	yellow	red	red	red	red	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	FC0662	red	red	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0639	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc I	CEP1032	yellow	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0498	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0655	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	C7838	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	C8729	red	red	red	red	red	yellow	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP0880	red	orange	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0101	red	orange	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0785	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0041	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C5394	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IV	CEP0185	red	red	red	yellow	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP1199	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	C5449	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0196	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0186	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IV	CEP1081	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0070	red	red	yellow	yellow	red	red	red	red	red	red	red	red
Bcc I	CEP0072	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0117	red	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc I	CEP0972	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1111	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0965	red	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0565	red	orange	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc V	CEP0087	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP1091	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0118	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	C8766	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1113	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0026	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	CEP0485	red	red	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0081	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc I	CEP0068	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0114	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0929	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0139	red	red	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc II	C9172	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	C6749	red	orange	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc II	CEP1116	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP0934	red	orange	orange	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0869	red	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP1079	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0951	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc IV	C6061	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0786	orange	orange	orange	orange	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0945	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0408	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1190	red	red	red	red	red	red	red	red	red	red	red	red
BPY	FC0433	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1012	red	orange	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	1114	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc II	CEP1235	orange	yellow	yellow	yellow	orange	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0591	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0777	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0969	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0769	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0686	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	FC0378	yellow	yellow	yellow	yellow	orange	range	orange	red	red	red	red	red
Bcc V	FC0465	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0615	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0357	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc VI	FC0353	red	orange	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc II	FC0102	red	orange	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	CEP0691	red	yellow	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc IIIa	CEP1161	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	FC0120	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	D0156	orange	yellow	yellow	yellow	red	red	red	orange	red	red	red	red
Bcc II	D0297	orange	yellow	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc VI	CEP1013	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	FC0362	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0961	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VIII	FC0964	yellow	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VIII	FC0965	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0628	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0768	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc II	CEP0699	orange	yellow	yellow	yellow	orange	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0986	red	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0455	red	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc V	C9710	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0215	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7637	red	red	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	C9287	red	red	red	red	yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIb	CEP0187	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0103	red	red	red	red	red	red	red	yellow	red	red	red	red
Bcc II	C8681	orange	yellow	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc V	C9371	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0235	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0240	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0102	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C5275	yellow	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0073	red	orange	yellow	yellow	red	red	red	yellow	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc V	CEP0084	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0617	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc II	CEP0169	red	yellow	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc V	C8952	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0146	red	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP0076	red	red	orange	yellow	red	red	orange	yellow	red	red	red	red
Bcc V	CEP0192	yellow	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0606	yellow	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc V	CEP0047	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0931	yellow	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc IV	CEP0404	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7263	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc I	C8536	red	red	orange	orange	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0233	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0142	red	red	orange	orange	red	red	red	red	red	red	red	red
Bcc II	D0056	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc I	CEP0074	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	CEP0243	yellow	yellow	yellow	yellow	red	red	red	yellow	red	red	red	red
Bcc II	D0155	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	FC0373	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP1140	red	red	yellow	yellow	red	red	red	red	red	red	red	red
Bcc V	D0278	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	C7329	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc V	FC0622	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP001	red	red	orange	orange	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP1236	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0601	red	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP0108	orange	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc V	CEP0175	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	CEP0990	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc II	CEP1000	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP0865	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP1064	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	CEP0958	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc I	CEP0834	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc IIIb	CEP0054	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0156	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0846	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0851	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0982	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0873	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IV	FC0473	red	orange	orange	yellow	red	red	red	red	red	red	red	red
Bcc VI	CEP0021	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IV	CEP0710	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1067	orange	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc VII	CEP0996	red	red	red	red	red	red	red	red	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	CEP1232	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0460	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP0743	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0660	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VI	CEP1011	red	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc II	CEP1129	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IIIa	FC1057	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC0457	red	red	red	orange	red	red	red	red	red	red	red	red
Bcc I	CEP1132	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc V	CEP1224	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	SQ004C	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP0630	red	orange	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc VIII	FC0974	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1119	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0703	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0706	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VII	FC0881	red	red	red	red	red	red	red	orange	red	red	red	red
Bcc IV	FC0772	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	CEP1129	orange	yellow	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc V	D0121	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IV	CEP0059	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	CEP0255	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC1104	red	red	red	red	red	red	red	red	red	red	red	red
Bcc VIII	FC0962	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VI	FC0380	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VIII	FC0963	yellow	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc II	D0445	red	orange	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0702	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0300	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0372	red	red	red	red	red	red	red	red	red	red	red	red
Bcc II	FC0898	orange	yellow	yellow	yellow	red	orange	yellow	yellow	red	red	red	red
Bcc VIII	FC0967	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	FC0506	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc VII	FC0623	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc VII	FC0882	orange	yellow	orange	orange	red	red	red	red	red	red	red	red
Bcc IIIb	FC0802	red	orange	yellow	yellow	red	red	yellow	yellow	red	red	red	red
Bcc IV	FC0503	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	FC0499	red	red	orange	yellow	red	red	yellow	yellow	red	red	red	red
BPY	FC0451	red	red	red	red	red	red	red	red	red	red	red	red
Bcc V	FC0463	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0635	red	red	red	red	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1184	red	red	red	red	red	red	red	red	red	red	red	red
Bcc I	FC1107	orange	yellow	yellow	yellow	red	red	orange	yellow	red	red	red	red
Bcc IV	FC0778	red	red	red	yellow	red	red	red	red	red	red	red	red
Bcc VIII	FC0968	orange	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1139	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow	red	red	red	red

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Maltose				Trehalose				Amygdaline			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VIII	FC0976	red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Bcc I	FC1108	red	red	red	red	red	red	yellow	yellow	red	red	red	red
Bcc I	CEP1151	red	red	orange	yellow	red	red	orange	yellow	red	red	red	red
Bcc VII	FC0767	red	red	orange	yellow	red	red	red	red	red	red	red	red
Bcc I	FC0649	yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Bcc V	FC0659	red	red	red	red	red	red	red	red	red	red	red	red
Positive control	see below	yellow	orange	yellow	yellow	yellow	yellow	yellow	yellow	red	red	red	red
Org free control		red	yellow	yellow	yellow	red	red	red	red	red	red	red	red
Org free control		red	red	red	red	red	red	red	red	red	red	red	red

Positive control Bcc strains

Carbohydrate	**Reference**
Maltose	LMG 17997
Trehalose	LMG 17588
Amygdaline	LMG 2161

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0717	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0506	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	CEP0126	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0824	red	red	red	red	red	red	red	red
Bcc I	CEP0060	red	red	red	red	red	orange	yellow	yellow
Bcc IV	CEP0469	red	red	red	red	red	red	red	red
Bcc V	CEP0999	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIb	CEP0762	red	red	red	red	red	red	yellow	yellow
Bcc IIIa	C4708	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0562	red	red	red	yellow	red	yellow	yellow	yellow
Bcc IV	CEP0112	red	red	red	red	red	red	yellow	yellow
Bcc IIIb	CEP0519	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	CEP0028	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	C8467	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0198	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	C9281	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0444	red	red	red	red	red	red	orange	yellow
Bcc II	CEP1006	red	red	red	red	red	red	red	red
Bcc I	CEP0533	red	red	red	red	red	red	orange	yellow
Bcc IIIa	CEP0507	red	red	red	red	orange	yellow	orange	yellow
Bcc II	CEP0773	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP1110	red	red	red	red	red	orange	yellow	yellow
Bcc IIIb	CEP0961	red	red	red	red	orange	yellow	orange	yellow
Bcc V	CEP0974	red	red	red	red	red	orange	yellow	yellow
Bcc VI	CEP0027	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	CEP0339	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0136	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0942	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc V	CEP0143	red	red	red	red	red	red	red	yellow
Bcc V	CEP0505	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	FC0427	red	red	red	red	red	red	red	red
Bcc II	CEP0181	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0750	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0663	red	red	red	red	red	red	red	red
Bcc II	C4356	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	CEP0242	red	red	red	red	red	orange	yellow	yellow
Bcc II	CEP0978	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	C6279	red	red	red	red	red	red	red	red
Bcc VII	CEP0516	red	red	red	red	yellow	orange	red	red
Bcc IIIa	CEP0755	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1114	red	red	red	red	orange	yellow	yellow	yellow
Bcc II	CEP0938	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0984	red	red	red	red	yellow	yellow	orange	yellow
Bcc IV	CEP0194	red	red	red	red	red	orange	yellow	yellow
Bcc IIIb	CEP0499	red	red	red	red	yellow	yellow	yellow	orange
Bcc IIIa	CEP0715	red	red	red	red	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IIIa	CEP0749	red	red	red	red	red	red	red	red
Bcc II	C7273	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	CEP0086	red	red	red	red	red	yellow	yellow	yellow
Bcc IV	CEP0176	red	red	red	red	red	red	red	red
Bcc IIIa	C4872	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0236	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0480	red	red	red	red	red	red	yellow	yellow
Bcc IIIa	CEP0787	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0957	red	red	red	red	red	red	red	orange
Bcc IV	CEP1025	red	red	red	red	red	red	red	orange
Bcc I	FC0449	red	red	red	red	red	red	red	orange
Bcc V	FC0464	red	red	red	red	red	red	orange	yellow
Bcc V	CEP0213	red	red	red	red	red	red	red	red
Bcc IV	CEP0500	red	red	red	red	red	orange	orange	yellow
Bcc IIIa	CEP0168	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IV	CEP0711	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	CEP0735	red	red	red	red	red	orange	yellow	yellow
Bcc I	CEP0069	red	red	red	red	red	orange	orange	yellow
Bcc II	C9346	red	red	red	red	red	red	red	yellow
Bcc IV	CEP0952	red	red	red	red	red	red	red	red
Bcc IV	CEP1075	red	red	red	red	red	red	red	red
Bcc II	CEP0503	red	red	red	red	red	red	yellow	yellow
Bcc IIIa	CEP0211	red	red	red	red	yellow	yellow	yellow	orange
Bcc II	CEP0493	orange	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc V	D0439	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0209	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc I	CEP0843	red	red	red	red	red	yellow	yellow	yellow
Bcc II	C7363	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	C7837	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	C8982	red	red	red	red	red	yellow	yellow	yellow
Bcc I	CEP0067	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0741	red	red	red	red	red	red	red	red
Bcc IIIb	C7946	red	red	red	orange	yellow	yellow	yellow	yellow
Bcc II	CEP1018	red	red	red	red	red	yellow	yellow	yellow
Bcc VI	CEP1007	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	CEP1014	orange	yellow	yellow	yellow	red	yellow	yellow	yellow
Bcc VI	CEP0766	yellow	yellow	yellow	yellow	red	yellow	yellow	yellow
Bcc VI	CEP1010	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	CEP1008	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0649	yellow	yellow	yellow	yellow	red	red	red	red
Bcc I	CEP1030	red	red	red	red	red	yellow	yellow	yellow
Bcc VIII	FC0972	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VIII	FC0970	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0616	red	red	red	red	red	red	red	red
Bcc VIII	FC0973	orange	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc VI	FC0346	red	red	red	red	yellow	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	FC0662	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0639	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP1032	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0498	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0655	red	red	red	red	orange	yellow	yellow	yellow
Bcc IV	C7838	red	red	red	red	red	red	red	red
Bcc I	C8729	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0880	red	red	red	red	red	red	yellow	yellow
Bcc I	CEP0101	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0785	red	red	red	red	red	red	red	red
Bcc V	CEP0041	red	red	red	red	red	red	red	red
Bcc II	C5394	red	red	red	red	orange	yellow	yellow	yellow
Bcc IV	CEP0185	red	red	red	red	red	orange	orange	yellow
Bcc IIIa	CEP1199	red	red	red	red	red	red	red	red
Bcc II	C5449	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0196	red	red	red	red	red	red	red	yellow
Bcc IIIa	CEP0186	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IV	CEP1081	red	red	red	red	red	red	red	orange
Bcc I	CEP0070	red	red	red	red	red	red	red	yellow
Bcc I	CEP0072	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0117	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0972	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIb	CEP1111	red	red	red	red	yellow	yellow	yellow	orange
Bcc II	CEP0965	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0565	red	orange	orange	yellow	yellow	yellow	yellow	red
Bcc V	CEP0087	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP1091	red	red	red	red	red	red	red	red
Bcc IV	CEP0118	red	red	red	red	yellow	orange	yellow	yellow
Bcc V	C8766	red	red	red	red	yellow	orange	yellow	yellow
Bcc IIIb	CEP1113	red	red	red	red	yellow	red	red	red
Bcc VI	CEP0026	red	red	red	red	orange	yellow	yellow	yellow
Bcc II	CEP0485	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0081	red	red	red	yellow	red	orange	yellow	yellow
Bcc I	CEP0068	red	red	red	red	red	red	yellow	yellow
Bcc IIIb	CEP0114	red	red	red	red	red	red	red	red
Bcc IV	CEP0929	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0139	red	red	red	red	orange	yellow	yellow	yellow
Bcc II	C9172	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	C6749	red	red	red	red	red	red	orange	yellow
Bcc II	CEP1116	red	red	red	red	red	orange	yellow	yellow
Bcc I	CEP0934	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	CEP0869	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	CEP1079	red	red	red	red	red	yellow	yellow	orange
Bcc IIIa	CEP0951	red	red	red	red	red	red	red	red
Bcc IV	C6061	red	red	red	red	red	red	red	red
Bcc II	CEP0786	red	orange	red	red	yellow	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0945	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0408	red	red	red	red	red	red	red	red
Bcc I	CEP1190	red	red	red	red	red	red	orange	yellow
BPY	FC0433	red	red	red	red	red	red	orange	yellow
Bcc VI	CEP1012	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	1114	red	red	red	red	red	yellow	yellow	yellow
Bcc II	CEP1235	red	red	red	red	yellow	yellow	yellow	orange
Bcc IIIa	CEP0591	red	red	red	red	red	yellow	yellow	yellow
Bcc IV	FC0777	red	red	red	red	red	red	red	yellow
Bcc VIII	FC0969	red	red	red	red	orange	yellow	yellow	yellow
Bcc IIIb	CEP0769	red	red	red	red	red	red	red	red
Bcc II	CEP0686	red	red	orange	orange	red	yellow	yellow	yellow
Bcc IIIb	FC0378	red	red	red	red	red	red	red	orange
Bcc V	FC0465	red	red	red	red	red	red	red	orange
Bcc I	CEP0615	red	red	red	red	red	red	red	red
Bcc II	FC0357	red	red	red	red	red	orange	yellow	yellow
Bcc VI	FC0353	red	red	red	red	red	yellow	yellow	yellow
Bcc II	FC0102	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	CEP0691	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP1161	red	red	red	red	red	red	red	red
Bcc IIIa	FC0120	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	D0156	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	D0297	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	CEP1013	red	red	red	red	red	orange	yellow	yellow
Bcc IV	FC0362	red	red	red	red	red	yellow	yellow	yellow
Bcc VIII	FC0961	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VIII	FC0964	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VIII	FC0965	red	red	red	red	orange	yellow	yellow	yellow
Bcc IIIb	CEP0628	red	orange	yellow	yellow	yellow	yellow	orange	red
Bcc IIIa	CEP0768	red	red	red	red	red	orange	orange	yellow
Bcc II	CEP0699	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIb	CEP0986	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	CEP0455	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	C9710	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0215	red	red	red	red	red	red	red	red
Bcc II	C7637	red	red	red	red	red	red	red	red
Bcc V	C9287	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0187	red	red	red	red	red	yellow	yellow	yellow
Bcc IV	CEP0103	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	C8681	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	C9371	red	red	red	red	orange	yellow	yellow	yellow
Bcc IV	CEP0235	red	red	red	red	red	red	orange	yellow
Bcc I	CEP0240	red	red	red	red	red	yellow	yellow	yellow
Bcc VII	CEP0102	red	red	red	red	red	yellow	yellow	yellow
Bcc II	C5275	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0073	red	red	red	red	red	orange	yellow	yellow

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc V	CEP0084	red	red	red	red	red	red	red	red
Bcc VII	CEP0617	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	CEP0169	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	C8952	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	CEP0146	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0076	red	red	red	red	red	orange	yellow	yellow
Bcc V	CEP0192	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP0606	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	CEP0047	red	orange	yellow	yellow	red	red	red	yellow
Bcc IIIa	CEP0931	red	orange	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IV	CEP0404	red	red	red	red	red	red	red	red
Bcc II	C7263	red	red	red	red	red	yellow	yellow	yellow
Bcc I	C8536	red	red	red	red	red	red	red	red
Bcc V	CEP0233	red	red	red	red	red	red	red	red
Bcc IV	CEP0142	red	red	red	red	red	orange	red	orange
Bcc II	D0056	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0074	red	red	red	red	red	orange	yellow	yellow
Bcc II	CEP0243	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	D0155	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	FC0373	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP1140	red	red	red	red	red	orange	yellow	yellow
Bcc V	D0278	red	red	red	red	red	red	red	red
Bcc II	C7329	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	FC0622	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP001	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	CEP1236	red	red	red	red	red	yellow	yellow	yellow
Bcc II	CEP0601	red	red	red	red	orange	yellow	yellow	yellow
Bcc II	CEP0108	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	CEP0175	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0990	red	red	red	red	red	red	red	red
Bcc II	CEP1000	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0865	red	red	red	red	red	red	red	red
Bcc IV	CEP1064	red	red	red	red	red	red	red	red
Bcc VII	CEP0958	red	red	red	red	yellow	yellow	red	red
Bcc I	CEP0834	red	red	red	red	red	orange	yellow	yellow
Bcc IIIb	CEP0054	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP0156	red	red	red	red	red	red	red	red
Bcc IV	CEP0846	red	red	red	red	red	red	red	red
Bcc IV	CEP0851	red	red	red	red	red	red	red	red
Bcc V	CEP0982	red	red	red	red	red	red	yellow	yellow
Bcc VI	CEP0873	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IV	FC0473	red	red	red	red	red	red	red	orange
Bcc VI	CEP0021	red	red	red	red	orange	yellow	yellow	yellow
Bcc IV	CEP0710	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1067	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VII	CEP0996	red	red	red	red	yellow	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	CEP1232	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	FC0460	red	red	red	red	red	red	red	red
Bcc VI	CEP0743	red	red	red	red	red	yellow	yellow	yellow
Bcc I	FC0660	red	red	red	red	red	red	yellow	yellow
Bcc VI	CEP1011	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	CEP1129	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	FC1057	red	red	red	red	red	red	red	red
Bcc I	FC0457	red	red	red	red	red	red	red	red
Bcc I	CEP1132	red	red	red	yellow	red	yellow	yellow	yellow
Bcc V	CEP1224	red	red	red	red	orange	yellow	yellow	yellow
Bcc V	SQ004C	red	red	red	red	red	red	yellow	yellow
Bcc II	CEP0630	red	red	red	red	red	red	yellow	yellow
Bcc VIII	FC0974	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP1119	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0703	red	red	red	red	orange	yellow	yellow	yellow
Bcc V	CEP0706	red	red	red	red	red	red	orange	yellow
Bcc VII	FC0881	red	red	red	red	red	red	yellow	yellow
Bcc IV	FC0772	red	red	red	red	red	red	orange	yellow
Bcc II	CEP1129	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	D0121	red	red	red	red	red	red	red	red
Bcc IV	CEP0059	red	red	red	red	red	red	red	red
Bcc V	CEP0255	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	FC1104	red	red	red	red	red	red	red	orange
Bcc VIII	FC0962	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	FC0380	red	red	red	red	red	red	red	red
Bcc VIII	FC0963	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	D0445	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP0702	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	CEP0300	red	red	red	red	red	red	red	orange
Bcc IIIb	FC0372	red	red	red	red	red	red	red	red
Bcc II	FC0898	red	red	red	red	red	yellow	yellow	yellow
Bcc VIII	FC0967	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	FC0506	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VII	FC0623	red	red	orange	orange	red	yellow	yellow	yellow
Bcc VII	FC0882	red	red	red	red	red	orange	yellow	yellow
Bcc IIIb	FC0802	red	red	red	red	red	orange	yellow	yellow
Bcc IV	FC0503	red	red	red	red	red	red	red	orange
Bcc IIIb	FC0499	red	red	red	red	red	red	yellow	yellow
BPY	FC0451	red	red	red	red	red	red	red	red
Bcc V	FC0463	red	red	red	red	red	red	yellow	yellow
Bcc IIIa	CEP0635	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1184	red	red	red	red	red	red	red	red
Bcc I	FC1107	red	red	red	red	red	red	yellow	yellow
Bcc IV	FC0778	red	red	red	red	red	red	red	red
Bcc VIII	FC0968	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP1139	red	orange	yellow	yellow	yellow	yellow	yellow	yellow

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Abutine				Cellobiose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VIII	FC0976	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	FC1108	red	red	red	red	red	red	red	red
Bcc I	CEP1151	red	red	red	red	red	red	yellow	yellow
Bcc VII	FC0767	red	red	red	red	red	red	yellow	yellow
Bcc I	FC0649	red	red	red	red	red	red	red	red
Bcc V	FC0659	red	red	red	red	red	red	orange	yellow
Positive control	see below	red	red	orange	yellow	yellow	yellow	yellow	yellow
Org free control		red	red	red	red	red	red	red	red
Org free control		red	red	red	red	red	red	red	red

Positive control Bcc strains

Carbohydrate	**Reference**
Arbutine	LMG 1222
Cellobiose	LMG 18888

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0717	red	red	red	yellow	red	red	red	red
Bcc IIIa	CEP0506	red	red	orange	yellow	red	yellow	yellow	yellow
Bcc V	CEP0126	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0824	red	red	red	red	red	orange	orange	orange
Bcc I	CEP0060	red	red	red	red	red	red	red	red
Bcc IV	CEP0469	red	red	red	red	red	red	red	red
Bcc V	CEP0999	red	red	red	red	red	red	orange	yellow
Bcc IIIb	CEP0762	red	red	red	red	red	red	red	red
Bcc IIIa	C4708	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0562	red	red	red	red	red	red	yellow	yellow
Bcc IV	CEP0112	red	red	red	yellow	red	red	orange	yellow
Bcc IIIb	CEP0519	red	yellow	yellow	yellow	yellow	yellow	yellow	orange
Bcc VI	CEP0028	red	red	red	red	red	yellow	yellow	yellow
Bcc II	C8467	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP0198	red	red	red	yellow	orange	yellow	yellow	yellow
Bcc II	C9281	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP0444	red	yellow	yellow	yellow	red	red	red	orange
Bcc II	CEP1006	red	red	red	red	red	red	red	red
Bcc I	CEP0533	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0507	red	orange	yellow	yellow	orange	orange	yellow	yellow
Bcc II	CEP0773	red	red	yellow	yellow	red	red	yellow	yellow
Bcc V	CEP1110	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0961	red	orange	yellow	yellow	red	red	orange	yellow
Bcc V	CEP0974	red	red	red	orange	red	red	red	red
Bcc VI	CEP0027	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0339	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0136	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIa	CEP0942	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	CEP0143	red	red	red	red	red	red	red	red
Bcc V	CEP0505	red	red	red	red	red	red	red	yellow
Bcc IIIa	FC0427	red	red	red	red	red	red	red	red
Bcc II	CEP0181	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0750	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0663	red	red	red	red	red	red	red	red
Bcc II	C4356	red	red	yellow	yellow	red	red	red	yellow
Bcc IIIa	CEP0242	red	yellow	yellow	yellow	red	red	yellow	yellow
Bcc II	CEP0978	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	C6279	red	red	red	red	red	red	red	red
Bcc VII	CEP0516	red	red	yellow	yellow	red	red	orange	yellow
Bcc IIIa	CEP0755	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1114	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc II	CEP0938	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIb	CEP0984	red	red	yellow	yellow	orange	orange	orange	orange
Bcc IV	CEP0194	red	red	red	yellow	red	red	orange	yellow
Bcc IIIb	CEP0499	red	red	yellow	yellow	red	orange	yellow	yellow
Bcc IIIa	CEP0715	red	red	red	red	red	red	red	red

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IIIa	CEP0749	red	red	red	red	red	red	red	red
Bcc II	C7273	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc V	CEP0086	red	red	red	red	red	red	orange	yellow
Bcc IV	CEP0176	red	red	red	red	red	orange	yellow	yellow
Bcc IIIa	C4872	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0236	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0480	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0787	red	orange	yellow	yellow	red	yellow	yellow	yellow
Bcc I	CEP0957	red	red	red	red	red	red	red	red
Bcc IV	CEP1025	red	red	red	red	red	red	red	red
Bcc I	FC0449	red	red	red	red	red	orange	orange	orange
Bcc V	FC0464	red	red	red	red	red	red	red	orange
Bcc V	CEP0213	red	red	red	red	red	red	red	red
Bcc IV	CEP0500	red	red	orange	yellow	red	orange	yellow	yellow
Bcc IIIa	CEP0168	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IV	CEP0711	red	yellow	yellow	yellow	yellow	orange	red	red
Bcc VI	CEP0735	red	red	red	red	red	red	yellow	yellow
Bcc I	CEP0069	red	red	red	red	red	red	orange	yellow
Bcc II	C9346	red	red	red	red	red	red	red	yellow
Bcc IV	CEP0952	red	red	red	red	red	red	red	red
Bcc IV	CEP1075	red	red	red	red	red	red	red	red
Bcc II	CEP0503	red	red	red	yellow	red	red	yellow	yellow
Bcc IIIa	CEP0211	red	yellow	yellow	yellow	yellow	yellow	orange	red
Bcc II	CEP0493	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	D0439	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0209	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc I	CEP0843	red	red	red	red	red	orange	yellow	yellow
Bcc II	C7363	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIa	C7837	red	red	yellow	yellow	red	orange	orange	yellow
Bcc II	C8982	red	red	red	yellow	red	yellow	yellow	yellow
Bcc I	CEP0067	red	red	red	red	red	yellow	yellow	yellow
Bcc V	CEP0741	red	red	red	red	red	red	red	red
Bcc IIIb	C7946	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc II	CEP1018	red	red	red	yellow	red	yellow	yellow	yellow
Bcc VI	CEP1007	red	red	red	red	red	yellow	yellow	yellow
Bcc VI	CEP1014	red	red	red	red	red	orange	orange	orange
Bcc VI	CEP0766	red	red	red	red	red	red	yellow	yellow
Bcc VI	CEP1010	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	CEP1008	red	red	red	red	red	orange	yellow	yellow
Bcc V	CEP0649	red	red	red	red	red	red	red	red
Bcc I	CEP1030	red	red	red	yellow	orange	yellow	yellow	yellow
Bcc VIII	FC0972	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VIII	FC0970	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0616	red	red	red	red	red	red	red	red
Bcc VIII	FC0973	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	FC0346	red	red	red	red	yellow	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	FC0662	red	red	red	red	red	orange	yellow	yellow
Bcc V	CEP0639	red	red	red	orange	red	orange	yellow	yellow
Bcc I	CEP1032	red	orange	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0498	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0655	red	yellow	yellow	yellow	orange	yellow	yellow	yellow
Bcc IV	C7838	red	red	red	red	red	red	red	red
Bcc I	C8729	Yellow	yellow	yellow	yellow	red	red	red	red
Bcc IIIa	CEP0880	red	red	yellow	yellow	red	red	yellow	yellow
Bcc I	CEP0101	red	red	red	yellow	red	red	orange	yellow
Bcc IIIa	CEP0785	red	red	red	red	red	red	red	red
Bcc V	CEP0041	red	red	red	red	red	red	red	red
Bcc II	C5394	red	orange	yellow	yellow	red	yellow	yellow	yellow
Bcc IV	CEP0185	red	red	yellow	yellow	red	orange	orange	yellow
Bcc IIIa	CEP1199	red	red	red	red	red	red	red	red
Bcc II	C5449	red	yellow	yellow	yellow	red	orange	yellow	yellow
Bcc V	CEP0196	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0186	red	red	red	red	orange	yellow	yellow	yellow
Bcc IV	CEP1081	red	red	red	red	red	red	red	red
Bcc I	CEP0070	red	red	red	red	red	red	yellow	yellow
Bcc I	CEP0072	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0117	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc I	CEP0972	red	red	red	red	red	red	yellow	yellow
Bcc IIIb	CEP1111	red	yellow	yellow	yellow	red	orange	orange	orange
Bcc II	CEP0965	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP0565	red	red	orange	yellow	red	yellow	yellow	yellow
Bcc V	CEP0087	red	red	red	red	red	red	red	yellow
Bcc IIIa	CEP1091	red	red	yellow	yellow	red	red	red	yellow
Bcc IV	CEP0118	red	red	red	red	red	red	yellow	yellow
Bcc V	C8766	red	red	red	red	red	orange	orange	yellow
Bcc IIIb	CEP1113	red	red	red	red	red	red	red	red
Bcc VI	CEP0026	red	red	red	red	red	yellow	yellow	yellow
Bcc II	CEP0485	red	red	yellow	yellow	red	orange	yellow	yellow
Bcc I	CEP0081	red	red	red	red	red	orange	yellow	yellow
Bcc I	CEP0068	red	red	red	red	red	red	red	yellow
Bcc IIIb	CEP0114	red	red	red	red	red	red	red	red
Bcc IV	CEP0929	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0139	red	red	orange	yellow	red	red	red	yellow
Bcc II	C9172	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	C6749	red	red	red	yellow	red	red	red	red
Bcc II	CEP1116	red	orange	yellow	yellow	red	yellow	yellow	yellow
Bcc I	CEP0934	red	red	yellow	yellow	red	orange	yellow	yellow
Bcc IIIa	CEP0869	red	red	red	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP1079	red	red	yellow	yellow	red	orange	orange	yellow
Bcc IIIa	CEP0951	red	red	red	yellow	red	red	red	red
Bcc IV	C6061	red	red	red	red	red	red	red	red
Bcc II	CEP0786	red	orange	yellow	yellow	red	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc IV	CEP0945	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0408	red	red	red	red	red	red	red	red
Bcc I	CEP1190	red	red	red	red	red	red	red	red
BPY	FC0433	red	red	red	red	red	red	red	red
Bcc VI	CEP1012	red	red	red	red	red	orange	yellow	yellow
Bcc IIIb	1114	red	red	red	yellow	red	red	yellow	yellow
Bcc II	CEP1235	yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0591	red	red	yellow	yellow	red	red	yellow	yellow
Bcc IV	FC0777	red	red	red	red	red	red	red	red
Bcc VIII	FC0969	red	red	red	red	orange	yellow	yellow	yellow
Bcc IIIb	CEP0769	red	red	red	red	red	red	red	red
Bcc II	CEP0686	red	red	orange	yellow	red	orange	yellow	yellow
Bcc IIIb	FC0378	red	orange	orange	orange	orange	orange	yellow	yellow
Bcc V	FC0465	red	red	red	red	red	red	red	orange
Bcc I	CEP0615	red	red	red	red	red	red	red	red
Bcc II	FC0357	red	red	yellow	yellow	red	red	red	orange
Bcc VI	FC0353	red	red	yellow	yellow	red	red	yellow	yellow
Bcc II	FC0102	red	red	red	red	red	yellow	yellow	yellow
Bcc II	CEP0691	red	orange	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP1161	red	red	red	red	red	red	red	red
Bcc IIIa	FC0120	red	orange	yellow	yellow	orange	yellow	yellow	yellow
Bcc II	D0156	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc II	D0297	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc VI	CEP1013	red	red	red	red	red	red	red	red
Bcc IV	FC0362	red	red	red	red	red	yellow	yellow	yellow
Bcc VIII	FC0961	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VIII	FC0964	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VIII	FC0965	red	red	red	red	red	yellow	yellow	yellow
Bcc IIIb	CEP0628	red	orange	yellow	yellow	orange	yellow	yellow	yellow
Bcc IIIa	CEP0768	yellow	yellow	yellow	yellow	red	red	orange	orange
Bcc II	CEP0699	yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIb	CEP0986	yellow	yellow	yellow	yellow	yellow	yellow	yellow	orange
Bcc II	CEP0455	red	yellow	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	C9710	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0215	red	red	red	red	red	red	red	red
Bcc II	C7637	red	red	red	yellow	red	red	red	red
Bcc V	C9287	red	red	red	red	red	red	red	red
Bcc IIIb	CEP0187	red	red	red	red	red	red	yellow	yellow
Bcc IV	CEP0103	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc II	C8681	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	C9371	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IV	CEP0235	yellow	yellow	yellow	yellow	yellow	yellow	orange	red
Bcc I	CEP0240	red	orange	yellow	yellow	orange	yellow	yellow	yellow
Bcc VII	CEP0102	red	red	yellow	yellow	red	red	red	yellow
Bcc II	C5275	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc I	CEP0073	red	red	yellow	yellow	red	orange	orange	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc V	CEP0084	red	red	red	red	red	red	red	red
Bcc VII	CEP0617	red	red	red	red	red	red	red	red
Bcc II	CEP0169	red	red	orange	yellow	red	yellow	yellow	yellow
Bcc V	C8952	red	red	red	red	red	red	red	red
Bcc IIIa	CEP0146	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc I	CEP0076	red	red	red	orange	red	orange	orange	yellow
Bcc V	CEP0192	red	red	red	red	red	red	orange	yellow
Bcc IIIa	CEP0606	red	red	red	yellow	yellow	yellow	yellow	yellow
Bcc V	CEP0047	red	red	red	red	red	red	yellow	yellow
Bcc IIIa	CEP0931	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IV	CEP0404	red	red	red	red	red	red	red	red
Bcc II	C7263	red	orange	yellow	yellow	yellow	yellow	yellow	yellow
Bcc I	C8536	red	yellow	yellow	yellow	red	red	red	red
Bcc V	CEP0233	red	red	red	red	red	red	red	red
Bcc IV	CEP0142	red	red	red	red	red	orange	orange	yellow
Bcc II	D0056	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	CEP0074	red	red	red	red	orange	yellow	yellow	yellow
Bcc II	CEP0243	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc II	D0155	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	FC0373	red	red	red	yellow	red	red	yellow	yellow
Bcc I	CEP1140	red	red	red	yellow	red	orange	yellow	yellow
Bcc V	D0278	red	red	red	red	red	red	red	red
Bcc II	C7329	red	orange	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	FC0622	red	red	red	red	red	red	red	red
Bcc IIIa	CEP001	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	CEP1236	red	red	red	red	red	red	red	red
Bcc II	CEP0601	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc II	CEP0108	red	orange	yellow	yellow	orange	yellow	yellow	yellow
Bcc V	CEP0175	red	red	yellow	yellow	yellow	yellow	yellow	yellow
Bcc I	CEP0990	red	red	red	red	red	red	red	yellow
Bcc II	CEP1000	red	red	red	red	red	red	yellow	yellow
Bcc V	CEP0865	red	red	red	red	red	red	red	red
Bcc IV	CEP1064	red	red	red	red	red	red	red	red
Bcc VII	CEP0958	red	red	red	red	red	red	red	red
Bcc I	CEP0834	red	red	red	yellow	red	red	orange	yellow
Bcc IIIb	CEP0054	red	red	red	red	red	red	red	orange
Bcc IIIa	CEP0156	red	red	red	red	red	red	red	red
Bcc IV	CEP0846	red	red	red	orange	red	red	red	red
Bcc IV	CEP0851	red	red	red	red	red	red	red	red
Bcc V	CEP0982	red	red	red	red	red	red	red	red
Bcc VI	CEP0873	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IV	FC0473	red	red	red	red	red	yellow	yellow	yellow
Bcc VI	CEP0021	red	red	red	red	red	yellow	yellow	yellow
Bcc IV	CEP0710	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1067	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc VII	CEP0996	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow

Appendix 3.10 (cont'd..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VII	CEP1232	red	red	red	yellow	red	red	red	red
Bcc I	FC0460	red	red	red	yellow	red	red	red	red
Bcc VI	CEP0743	red	red	red	red	red	red	orange	yellow
Bcc I	FC0660	red	red	red	red	red	red	red	red
Bcc VI	CEP1011	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc II	CEP1129	red	red	yellow	yellow	orange	yellow	yellow	yellow
Bcc IIIa	FC1057	red	red	red	red	red	red	red	red
Bcc I	FC0457	red	red	red	red	red	red	red	red
Bcc I	CEP1132	red	red	red	yellow	red	orange	yellow	yellow
Bcc V	CEP1224	red	red	red	red	yellow	yellow	yellow	yellow
Bcc V	SQ004C	red	red	red	red	red	red	red	red
Bcc II	CEP0630	red	red	yellow	yellow	red	red	yellow	yellow
Bcc VIII	FC0974	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP1119	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Bcc IIIa	CEP0703	red	red	red	orange	red	yellow	yellow	yellow
Bcc V	CEP0706	red	red	red	red	red	red	red	red
Bcc VII	FC0881	red	red	red	yellow	red	red	red	red
Bcc IV	FC0772	red	red	red	red	red	red	red	red
Bcc II	CEP1129	red	orange	yellow	yellow	yellow	yellow	yellow	yellow
Bcc V	D0121	red	red	red	red	red	red	red	red
Bcc IV	CEP0059	red	red	red	red	red	red	red	red
Bcc V	CEP0255	red	red	red	red	red	red	red	red
Bcc I	FC1104	red	red	red	red	red	red	red	red
Bcc VIII	FC0962	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VI	FC0380	red	red	red	red	red	red	red	red
Bcc VIII	FC0963	red	red	red	red	yellow	yellow	yellow	yellow
Bcc II	D0445	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc IIIa	CEP0702	red	red	red	red	red	red	red	yellow
Bcc IIIa	CEP0300	red	red	red	yellow	red	red	red	red
Bcc IIIb	FC0372	red	red	red	red	red	red	red	red
Bcc II	FC0898	red	orange	yellow	yellow	orange	yellow	yellow	yellow
Bcc VIII	FC0967	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIa	FC0506	red	red	red	red	yellow	yellow	yellow	yellow
Bcc VII	FC0623	red	red	red	orange	red	red	red	orange
Bcc VII	FC0882	red	red	red	yellow	orange	yellow	orange	red
Bcc IIIb	FC0802	red	red	yellow	yellow	red	orange	orange	orange
Bcc IV	FC0503	red	red	yellow	yellow	red	red	red	red
Bcc IIIb	FC0499	red	red	yellow	yellow	red	red	red	yellow
BPY	FC0451	red	red	yellow	yellow	red	yellow	yellow	yellow
Bcc V	FC0463	red	red	red	red	red	red	red	yellow
Bcc IIIa	CEP0635	red	red	red	red	red	red	red	red
Bcc IIIb	CEP1184	red	red	red	red	red	red	red	red
Bcc I	FC1107	red	red	orange	yellow	orange	yellow	orange	orange
Bcc IV	FC0778	red	red	red	red	red	red	yellow	yellow
Bcc VIII	FC0968	red	red	red	red	yellow	yellow	yellow	yellow
Bcc IIIb	CEP1139	red	yellow	yellow	yellow	yellow	yellow	yellow	yellow

Appendix 3.10 (cont`d..): Colour development recordings of various Bcc strains due to carbohydrate oxidation

		Trehalose (duplicate)				Maltose (duplicate)			
Strain	**Reference**	**48hr**	**72hr**	**96hr**	**5 day**	**48hr**	**72hr**	**96hr**	**5 day**
Bcc VIII	FC0976	red	red	red	red	yellow	yellow	yellow	yellow
Bcc I	FC1108	red	orange	yellow	yellow	red	red	red	red
Bcc I	CEP1151	red	red	orange	yellow	red	orange	yellow	yellow
Bcc VII	FC0767	red	red	red	red	red	red	red	red
Bcc I	FC0649	red	red	red	red	red	red	red	red
Bcc V	FC0659	red	red	red	red	red	red	red	orange
Positive control	see below	yellow	yellow	yellow	yellow	red	orange	yellow	yellow
Org free control		red	red	red	red	red	red	red	red
Org free control		red	red	red	red	red	red	red	red

Positive control Bcc strains

Carbohydrate	**Reference**
Trehalose	LMG 17588
Maltose	LMG 17997

Appendix 3.11a: Positive and negative reactions for hydrolysis of indoxylic substrates on chromogenic strips for Bcc and *P. aeruginosa* and four control strains

	Strain and Reference					
	E.coli	*K. pneumoniae*	*B. cepacia*	*P. aeruginosa*	*B. cepacia*	*B. cepacia*
Substrate	NCTC 10418	NCTC 10896	LMG 1222	NCTC 10662	LMG 1222	LMG 2161
X NAGlu	-	-	+	-	+	+
Magenta NAGlu	-	-	+	-	+	+
Rose NAGlu	-	+	+	-	+	+
X NAGal	-	-	+	-	+	-
X ßD GAL	+	+	-	-	-	-
Magenta ß GAL	+	+	-	-	-	-
Rose ßD GAL	+	+	-	-	-	-
Blue ßD GAL	+	+	-	-	-	-
4 Cl 3 I ßDGAL	+	+	-	-	-	-
F ßD GAL	+	+	+	-	+	+
8 HQ ßD GAL	-	-	-	-	-	-
Y ßD GAL	-	-	+	-	+	+
5 I 3 I ßD GAL	-	-	-	-	-	-
Green ßD GAL	-	+	-	-	-	-
X αD GAL	+	-	-	-	-	+
Magenta αD GAL	-	-	-	-	-	+
Rose αD GAL	-	-	-	-	-	+
Blue αD GAL	+	-	-	-	-	+
X ßD GLU	+	-	-	-	-	-
Magenta ßD GLU	-	+	+	-	+	+
Rose ßD GLU	-	+	+	-	+	+
8 HQ ßDGLU	-	-	+	-	+	+
Y ßD GLU	-	-	+	-	+	+
X ßD cellobioside	-	+	+	-	+	+
Rose ßD cellobioside	-	+	+	-	+	+
X αD GLU	-	-	-	-	-	+
X ßD GUR	+	-	-	-	-	-
Magenta ßD GUR	-	-	-	-	-	-
Rose ßD GUR	+	-	-	-	-	-
Blue ßD GUR	+	-	-	-	-	-
Y ßD GUR	-	-	-	-	-	-
X αD Mann	-	-	+	-	+	+
Rose αD Mann	-	-	+	-	+	+
Blue αD Mann	-	-	-	-	-	+
X ßD Xyl	-	+	+	-	+	+
X ßD fucoside	-	-	-	-	-	-
X ßL fucoside	-	-	-	-	-	-
X P	-	-	+	-	+	+
Magenta P	+	+	+	-	+	+
Rose P	-	-	+	-	+	+
Y P	+	+	+	-	+	+
X sulfate	-	-	-	-	-	-
Magenta sulfate	-	-	-	-	-	-
Y sulfate	-	-	-	-	-	-
X acetate	-	-	+	+	+	+
Y acetate	-	-	+	-	+	+
X butyrate	-	-	+	+	+	+
X caprylate	-	-	+	+	+	+
Magenta caprylate	-	-	+	+	+	+
Rose caprylate	-	-	+	-	+	+
Blue D ala	-	-	-	-	-	-
Blue L ala	-	-	+	-	+	+
Blue L leu	-	-	-	-	-	-

Appendix 3.11a (cont..): Positive and negative reactions for hydrolysis of indoxylic substrates on chromogenic strips for Bcc and *P. aeruginosa* and four control strains

	Strain and Reference				
	B. cenocepacia	*B. cenocepacia*	*B. multivorans*	*B. multivorans*	*B. stabilis*
Substrate	LMG 16654	LMG 16656	LMG 13010	LMG 16660	LMG 14086
X NAGlu	+	+	+	+	+
Magenta NAGlu	+	+	+	+	+
Rose NAGlu	+	+	+	+	+
X NAGal	+	+	-	-	-
X ßD GAL	-	-	-	-	-
Magenta ß GAL	-	-	-	-	-
Rose ßD GAL	-	-	+	+	-
Blue ßD GAL	-	-	+	-	-
4 CI 3 I ßDGAL	-	-	-	-	+
F ßD GAL	+	+	+	+	+
8 HQ ßD GAL	-	-	-	-	-
Y ßD GAL	+	+	+	+	+
5 I 3 I ßD GAL	-	-	-	-	-
Green ßD GAL	-	-	-	-	-
X αD GAL	-	-	-	-	+
Magenta αD GAL	-	-	-	-	+
Rose αD GAL	-	-	-	-	+
Blue αD GAL	-	-	-	-	+
X ßD GLU	-	-	-	-	-
Magenta ßD GLU	+	+	+	+	+
Rose ßD GLU	+	+	+	+	+
8 HQ ßDGLU	+	+	+	+	+
Y ßD GLU	+	+	+	+	+
X ßD cellobioside	+	+	+	+	+
Rose ßD cellobioside	+	+	+	+	+
X αD GLU	+	+	+	-	-
X ßD GUR	-	-	-	-	-
Magenta ßD GUR	-	-	-	-	-
Rose ßD GUR	-	-	-	-	-
Blue ßD GUR	-	-	-	-	-
Y ßD GUR	-	-	-	-	-
X αD Mann	+	+	+	+	+
Rose αD Mann	+	+	+	+	+
Blue αD Mann	-	-	-	-	-
X ßD Xyl	+	+	+	+	-
X ßD fucoside	-	-	-	-	-
X ßL fucoside	-	-	-	-	-
X P	+	+	+	+	+
Magenta P	+	+	+	+	+
Rose P	+	+	+	+	+
Y P	+	+	+	+	+
X sulfate	-	-	-	-	-
Magenta sulfate	-	-	-	-	-
Y sulfate	-	-	-	-	-
X acetate	+	+	+	+	+
Y acetate	+	+	+	+	+
X butyrate	+	+	+	+	+
X caprylate	+	+	+	+	+
Magenta caprylate	+	+	+	+	+
Rose caprylate	+	+	+	+	+
Blue D ala	-	-	-	-	-
Blue L ala	+	+	+	+	+
Blue L leu	-	-	-	-	-

Appendix 3.11a (cont..): Positive and negative reactions for hydrolysis of indoxylic substrates on chromogenic strips for Bcc and *P. aeruginosa* and four control strains

	Strain and Reference				
	B. stabilis	*B. vietnamiensis*	*B. vietnamiensis*	*B. ambifaria*	*B. dolosa*
Substrate	LMG 14294	LMG 10929	LMG 16232	LMG 11351	LMG 18941
X NAGlu	-	-	-	+	+
Magenta NAGlu	-	-	-	+	+
Rose NAGlu	-	-	-	+	+
X NAGal	-	-	-	+	-
X ßD GAL	-	-	-	+	+
Magenta ß GAL	-	-	-	+	+
Rose ßD GAL	-	-	-	+	+
Blue ßD GAL	-	-	-	+	+
4 Cl 3 I ßDGAL	-	-	-	+	+
F ßD GAL	-	-	+	+	+
8 HQ ßD GAL	-	-	-	-	+
Y ßD GAL	-	-	+	+	+
5 I 3 I ßD GAL	-	-	-	-	+
Green ßD GAL	-	-	-	+	+
X αD GAL	-	-	-	+	+
Magenta αD GAL	-	-	-	+	-
Rose αD GAL	-	-	-	-	-
Blue αD GAL	-	-	-	-	-
X ßD GLU	-	-	-	-	-
Magenta ßD GLU	-	+	+	+	+
Rose ßD GLU	-	+	+	+	+
8 HQ ßDGLU	-	+	-	+	-
Y ßD GLU	-	-	-	+	+
X ßD cellobioside	-	+	-	+	+
Rose ßD cellobioside	-	-	-	+	+
X αD GLU	-	-	-	+	+
X ßD GUR	-	-	-	-	-
Magenta ßD GUR	-	-	-	-	-
Rose ßD GUR	-	-	-	-	-
Blue ßD GUR	-	-	-	-	-
Y ßD GUR	-	-	-	-	-
X αD Mann	-	-	-	-	-
Rose αD Mann	-	-	-	-	-
Blue αD Mann	-	-	-	-	-
X ßD Xyl	-	+	-	+	-
X ßD fucoside	-	+	-	+	-
X ßL fucoside	-	-	-	-	-
X P	+	+	+	+	+
Magenta P	-	+	+	+	+
Rose P	+	+	+	+	+
Y P	+	+	+	+	+
X sulfate	-	-	-	-	-
Magenta sulfate	-	-	-	-	-
Y sulfate	-	-	-	-	-
X acetate	+	+	+	+	+
Y acetate	+	+	+	+	+
X butyrate	+	+	+	+	+
X caprylate	+	+	+	+	+
Magenta caprylate	+	+	+	+	+
Rose caprylate	+	-	+	-	+
Blue D ala	-	-	-	-	-
Blue L ala	+	-	-	-	-
Blue L leu	-	-	-	-	-

Appendix 3.11a (cont..): Positive and negative reactions for hydrolysis of indoxylic substrates on chromogenic strips for Bcc and *P. aeruginosa* and four control strains

	Strain and Reference				
	B. dolosa	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Substrate	LMG 18942	Mb 2742	Mb 2749	Mb 2772	Mb 2775
X NAGlu	+	-	-	-	-
Magenta NAGlu	+	-	-	-	-
Rose NAGlu	+	-	-	-	-
X NAGal	+	-	-	-	-
X ßD GAL	-	-	-	-	-
Magenta ß GAL	-	-	-	-	-
Rose ßD GAL	-	-	-	-	-
Blue ßD GAL	-	-	-	-	-
4 CI 3 I ßDGAL	-	-	-	-	-
F ßD GAL	+	-	-	-	-
8 HQ ßD GAL	-	-	-	-	-
Y ßD GAL	+	-	+	-	-
5 I 3 I ßD GAL	-	-	-	-	-
Green ßD GAL	-	-	-	-	-
X αD GAL	-	-	-	-	-
Magenta αD GAL	-	-	-	-	-
Rose αD GAL	-	-	-	-	-
Blue αD GAL	-	-	-	-	-
X ßD GLU	-	-	-	-	-
Magenta ßD GLU	+	-	-	-	-
Rose ßD GLU	+	-	-	-	-
8 HQ ßDGLU	-	-	-	-	-
Y ßD GLU	+	-	-	-	-
X ßD cellobioside	+	-	-	-	-
Rose ßD cellobioside	+	-	-	-	-
X αD GLU	-	-	-	-	-
X ßD GUR	-	-	-	-	-
Magenta ßD GUR	-	-	-	-	-
Rose ßD GUR	-	-	-	-	-
Blue ßD GUR	-	-	-	-	-
Y ßD GUR	-	-	-	-	-
X αD Mann	-	-	-	-	-
Rose αD Mann	-	-	-	-	-
Blue αD Mann	-	-	-	-	-
X ßD Xyl	+	-	-	-	-
X ßD fucoside	-	-	-	-	-
X ßL fucoside	-	-	-	-	-
X P	+	-	+	+	+
Magenta P	+	-	-	-	-
Rose P	+	-	-	-	-
Y P	+	-	-	-	-
X sulfate	-	-	-	-	-
Magenta sulfate	-	-	-	-	-
Y sulfate	-	-	-	-	-
X acetate	+	+	+	+	-
Y acetate	+	+	-	+	-
X butyrate	+	+	+	+	-
X caprylate	+	+	+	+	-
Magenta caprylate	+	+	+	+	+
Rose caprylate	-	+	-	+	-
Blue D ala	-	-	-	-	-
Blue L ala	-	+	-	+	+
Blue L leu	-	-	-	-	-

Appendix 3.11a (cont..): Positive and negative reactions for hydrolysis of indoxylic substrates on chromogenic strips for Bcc and *P. aeruginosa* and four control strains

	Strain and Reference				
	P. aeruginosa	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
Substrate	BSAC PS1	PS10	PS20	PS30	PS40
X NAGlu	-	-	-	-	-
Magenta NAGlu	-	-	-	-	-
Rose NAGlu	-	-	-	-	-
X NAGal	-	-	-	-	-
X ßD GAL	-	-	-	-	-
Magenta ß GAL	-	-	-	-	-
Rose ßD GAL	-	-	-	-	-
Blue ßD GAL	-	-	-	-	-
4 CI 3 I ßDGAL	-	-	-	-	-
F ßD GAL	-	-	-	-	-
8 HQ ßD GAL	-	-	-	-	-
Y ßD GAL	-	-	-	-	-
5 I 3 I ßD GAL	-	-	-	-	-
Green ßD GAL	-	-	-	-	-
X αD GAL	-	-	-	-	-
Magenta αD GAL	-	-	-	-	-
Rose αD GAL	-	-	-	-	-
Blue αD GAL	-	-	-	-	-
X ßD GLU	-	-	-	-	-
Magenta ßD GLU	-	-	-	-	-
Rose ßD GLU	-	-	-	-	-
8 HQ ßDGLU	-	-	-	-	-
Y ßD GLU	-	-	-	-	-
X ßD cellobioside	-	-	-	-	-
Rose ßD cellobioside	-	-	-	-	-
X αD GLU	-	-	-	-	-
X ßD GUR	-	-	-	-	-
Magenta ßD GUR	-	-	-	-	-
Rose ßD GUR	-	-	-	-	-
Blue ßD GUR	-	-	-	-	-
Y ßD GUR	-	-	-	-	-
X αD Mann	-	-	-	-	-
Rose αD Mann	-	-	-	-	-
Blue αD Mann	-	-	-	-	-
X ßD Xyl	-	-	-	-	-
X ßD fucoside	-	-	-	-	-
X ßL fucoside	-	-	-	-	-
X P	-	+	-	-	-
Magenta P	-	-	-	-	-
Rose P	-	-	-	-	-
Y P	-	-	-	-	-
X sulfate	-	-	-	-	-
Magenta sulfate	-	-	-	-	-
Y sulfate	-	-	-	-	-
X acetate	+	+	+	-	+
Y acetate	+	+	+	-	-
X butyrate	+	+	+	+	+
X caprylate	+	+	+	+	+
Magenta caprylate	+	+	+	+	+
Rose caprylate	-	+	+	-	-
Blue D ala	-	-	-	-	-
Blue L ala	+	+	+	-	-
Blue L leu	-	-	-	-	-

Appendix 3.11a (cont..): Positive and negative reactions for hydrolysis of indoxylic substrates on chromogenic strips for Bcc and *P. aeruginosa* and four control strains

	Strain and Reference		
	P. aeruginosa	*P. aeruginosa*	*P. aeruginosa*
Substrate	PS50	NCTC 6749	NCTC 10332
X NAGlu	-	-	-
Magenta NAGlu	-	-	-
Rose NAGlu	-	-	-
X NAGal	-	-	-
X ßD GAL	-	-	-
Magenta ß GAL	-	-	-
Rose ßD GAL	-	-	-
Blue ßD GAL	-	-	-
4 CI 3 I ßDGAL	-	-	-
F ßD GAL	-	-	-
8 HQ ßD GAL	-	-	-
Y ßD GAL	-	-	-
5 I 3 I ßD GAL	-	-	-
Green ßD GAL	-	-	-
X αD GAL	-	-	-
Magenta αD GAL	-	-	-
Rose αD GAL	-	-	-
Blue αD GAL	-	-	-
X ßD GLU	-	-	-
Magenta ßD GLU	-	-	-
Rose ßD GLU	-	-	-
8 HQ ßDGLU	-	-	-
Y ßD GLU	-	-	-
X ßD cellobioside	-	-	-
Rose ßD cellobioside	-	-	-
X αD GLU	-	-	-
X ßD GUR	-	-	-
Magenta ßD GUR	-	-	-
Rose ßD GUR	-	-	-
Blue ßD GUR	-	-	-
Y ßD GUR	-	-	-
X αD Mann	-	-	-
Rose αD Mann	-	-	-
Blue αD Mann	-	-	-
X ßD Xyl	-	-	-
X ßD fucoside	-	-	-
X ßL fucoside	-	-	-
X P	-	-	-
Magenta P	-	-	-
Rose P	-	-	-
Y P	-	-	-
X sulfate	-	-	-
Magenta sulfate	-	-	-
Y sulfate	-	-	-
X acetate	-	+	+
Y acetate	-	-	-
X butyrate	-	+	+
X caprylate	-	+	+
Magenta caprylate	+	+	+
Rose caprylate	-	-	+
Blue D ala	-	-	-
Blue L ala	-	-	+
Blue L leu	-	-	-

Appendix 3.11b: Full indoxylic substrate names from chromogenic strips

Abbreviation	Substrate
X NAGlu	5-bromo-4-chloro-3-indolyl-N-acetyl-β-D-glucosaminide
Magenta NAGlu	5-bromo-6-chloro-3-indolyl-N-acetyl-β-D-glucosaminide
Rose NAGlu	6-chloro-3-indolyl-N-acetyl-β-D-glucosamide
X NAGal	5-bromo-4-chloro-3-indolyl-N-acetyl-β-D-galactosaminide
X ßD GAL	5-bromo-4-chloro-3-indolyl-β-D-galactoside
Magenta ß GAL	5-bromo-6-chloro-3-indolyl-β-D-galactoside
Rose ßD GAL	6-chloro-3-indolyl-β-D-galactoside
Blue ßD GAL	5-bromo-3-indolyl-β-D-galactoside
4 CI 3 I ßDGAL	4-chloro-3-indolyl-β-D-galactoside
F ßD GAL	6-fluoro-3-indolyl-β-D-galactoside
8 HQ ßD GAL	8-hydroxyquinoline-β-D-galactoside
Y ßD GAL	3-indolyl-β-D-galactoside
5 I 3 I ßD GAL	5-iodo-3-indolyl-β-D-galactoside
Green ßD GAL	N-methylindolyl-β-D-galactoside
X αD GAL	5-bromo-4-chloro-3-indolyl-α-D-galactoside
Magenta αD GAL	5-bromo-6-chloro-3-indolyl-α-D-galactoside
Rose αD GAL	6-chloro-3-indolyl-α-D-galactoside
Blue αD GAL	5-bromo-3-indolyl-α-D-galactoside
X ßD GLU	5-bromo-4-chloro-3-indolyl-β-D-glucoside
Magenta ßD GLU	5-bromo-6-chloro-3-indolyl-β-D-glucoside
Rose ßD GLU	6-chloro-3-indolyl-β-D-glucoside
8 HQ ßDGLU	8-hydroxyquinoline-β-D-glucoside
Y ßD GLU	3-indolyl-β-D-glucoside
X ßD cellobioside	5-bromo-4-chloro-3-indolyl-β-D-cellobioside
Rose ßD cellobioside	6-chloro-3-indolyl-β-D-cellobioside
X αD GLU	5-bromo-4-chloro-3-indolyl-α-D-glucoside
X ßD GUR	5-bromo-4-chloro-3-indolyl-β-D-glucuronide
Magenta ßD GUR	5-bromo-6-chloro-3-indolyl-β-D-glucuronide
Rose ßD GUR	6-chloro-3-indolyl-β-D-glucuronide
Blue ßD GUR	5-bromo-3-indolyl-β-D-glucuronide
Y ßD GUR	3-indolyl-β-D-glucuronide
X αD Mann	5-bromo-4-chloro-3-indolyl-α-D-mannoside
Rose αD Mann	6-chloro-3-indolyl-α-D-mannoside
Blue αD Mann	5-bromo-3-indoxyl-α-D-mannoside
X ßD Xyl	5-bromo-4-chloro-3-indolyl-β-D-xyloside
X ßD fucoside	5-bromo-4-chloro-3-indolyl-β-D-fucoside
X ßL fucoside	5-bromo-4-chloro-3-β-L-fucoside
X P	5-bromo-4-chloro-3-indolyl-phosphate
Magenta P	5-bromo-6-chloro-3-indolyl-phosphate
Rose P	6-chloro-3-indolyl-phosphate
Y P	3-indolyl-phosphate
X sulfate	5-bromo-4-chloro-3-indolyl-sulphate
Magenta sulfate	5-bromo-6-chloro-3-indolyl-sulfate
Y sulfate	3-indolyl-sulphate
X acetate	5-bromo-4-chloro-3-indolyl-acetate
Y acetate	3-indoxyl-3-acetate
X butyrate	5-bromo-4-chloro-3-indolyl-butyrate
X caprylate	5-bromo-4-chloro-3-indolyl-caprylate
Magenta caprylate	5-bromo-6-chloro-3-indolyl-caprylate
Rose caprylate	6-chloro-indolyl-caprylate
Blue D ala	5-bromo-3-indolamine-D-alanine
Blue L ala	5-bromo-3-indolamine-L-alanine
Blue L leu	5-bromo-3-indolamine-L-leucine

Appendix 4.1: Bcc strains selected from BCCM reference panel for synergy studies with alafosfalin

No.	Strain	Reference
Bcc 1	*B. cenocepacia*	LMG 16654
Bcc 2	*B. cenocepacia*	LMG 16656
Bcc 3	*B. cenocepacia*	LMG 16659
Bcc 4	*B. cepacia*	LMG 17997
Bcc 5	*B. cepacia*	LMG 18821
Bcc 6	*B. multivorans*	LMG 18826
Bcc 7	*B. cenocepacia*	LMG 18827
Bcc 8	*B. cenocepacia*	LMG 18828
Bcc 9	*B. cenocepacia*	LMG 18829
Bcc 10	*B. cenocepacia*	LMG 18830
Bcc 11	*B. cenocepacia*	LMG 18832
Bcc 12	*B. cenocepacia*	LMG 18863
Bcc 13	*B. multivorans*	LMG 13010
Bcc 14	*B. multivorans*	LMG 16660
Bcc 15	*B. multivorans*	LMG 18822
Bcc 16	*B. stabilis*	LMG 14294
Bcc 17	*B. stabilis*	LMG 18870
Bcc 18	*B. vietnamiensis*	LMG 16232
Bcc 19	*B. vietnamiensis*	LMG 18835

Appendix 4.2a: Fractional Inhibitory Concentrations (FICs) for all antibiotic combinations tested against Bcc strains

	Antibiotic combinations																			
	ALA/CAZ		ALA/MER		ALA/TAZ		ALA/TIM		ALA/CIP		ALA/CEF		ALA/AZT		ALA/TOB		TOB/CAZ-A		TOB/CAZ+A	
	Run		Run		Run		Run		Run		Run		Run		Run		Run		Run	
Organism	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Bcc 1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.53	0.63	1	1
Bcc 2	2	0.53	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.53	0.63	1	1
Bcc 3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bcc 4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1
Bcc 5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1
Bcc 6	2	2	0.53	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bcc 7	0.53	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.53	0.56	1	1
Bcc 8	0.53	2	0.38	0.31	2	2	2	0.16	2	2	2	2	2	2	2	2	0.63	0.63	1	1
Bcc 9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bcc 10	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bcc 11	2	0.63	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bcc 12	2	2	1	0.53	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
Bcc 13	0.53	0.16	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1
Bcc 14	0.53	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.75	1	1	1
Bcc 15	0.06	0.25	0.38	0.38	2	0.19	0.28	0.16	2	2	0.31	0.31	0.19	0.75	2	2	0.53	0.53	0.75	0.53
Bcc 16	0.06	0.28	0.38	2	0.13	0.19	0.38	2	2	2	0.31	0.75	0.16	0.5	2	2	2	2	2	2
Bcc 17	0.16	0.28	2	0.56	2	2	2	2	2	2	2	2	0.53	2	2	2	0.63	0.56	0.28	0.56
Bcc 18	2	0.38	0.56	0.63	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
Bcc 19	2	0.63	0.56	0.28	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.53
CONTROL	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Key:
ALA, alafosfalin; CAZ, Ceftazidime; MER, meropenem; TAZ, piperacillin/tazobactam; TIM, Ticarcillin/clavulanic acid;
CIP, ciprofloxacin; CEF, cefsulodin; AZT, aztreonam; TOB, tobramycin; TOB/CAZ+A, tobramycin and ceftazidime with alafosfalin;
TOB/CAZ-A, tobramycin and ceftazidime

Appendix 4.2b: Fractional Inhibitory Concentrations (FICs) for all antibiotic combinations tested against *P. aeruginosa*

	Antibiotic combinations																			
	ALA/CAZ		ALA/MER		ALA/TAZ		ALA/TIM		ALA/CIP		ALA/CEF		ALA/AZT		ALA/TOB		TOB/CAZ-A		TOB/CAZ+A	
	Run		Run		Run		Run		Run		Run		Run		Run		Run		Run	
Organism	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
PAE1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.75	0.75	2	2
PAE2	2	2	2	2	2	2	2	2	2	2	2	2	0.53	0.53	1	0.56	2	2	0.75	0.75
PAE3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.09	0.06	0.31	0.56
PAE4	0.75	2	0.28	0.53	2	2	0.75	1	2	2	2	2	2	2	0.75	0.63	2	2	2	2
PAE5	2	0.25	0.63	2	0.28	2	0.53	0.28	2	2	2	2	0.53	2	2	2	2	2	2	1
PAE6	2	2	2	2	0.53	0.63	2	2	2	2	2	2	2	2	2	2	2	2	2	2
PAE7	2	2	2	2	2	0.75	2	2	2	2	2	2	2	2	2	2	0.56	0.5	0.09	0.38
PAE8	2	2	0.53	2	2	2	2	2	2	2	2	2	2	2	2	2	0.63	0.63	0.63	0.31
PAE9	2	2	0.53	2	2	2	0.53	0.53	2	2	2	2	2	2	2	2	0.75	0.75	0.28	0.53
PAE10	2	2	0.53	0.53	2	2	2	2	2	2	2	2	0.38	0.75	2	2	2	0.75	1	2
PAE11	2	2	2	2	2	2	2	2	2	2	2	2	2	1	0.53	2	2	2	0.53	0.28
PAE12	2	2	2	2	0.53	2	2	2	2	2	2	2	0.53	2	2	2	0.56	0.53	0.63	0.56
PAE13	2	2	2	2	2	2	2	2	2	2	2	2	0.063	0.06	0.63	2	2	2	0.38	0.31
PAE14	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.75	0.57	0.06	0.09
PAE15	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	0.28
PAE16	2	0.16	2	2	2	2	2	2	2	2	2	2	0.56	2	2	2	2	2	2	2
PAE17	2	2	2	2	0.28	0.75	2	2	2	2	2	2	0.56	0.75	2	2	2	2	2	2
PAE18	2	2	2	2	2	2	2	2	2	2	0.28	2	2	2	2	2	2	2	2	2
PAE19	2	2	2	2	2	2	0.53	0.53	2	2	0.53	2	2	2	2	2	2	2	0.75	0.75
CONTROL	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Key:

PAE, *P. aeruginosa* ;ALA, alafosfalin; CAZ, Ceftazidime; MER, meropenem; TAZ, piperacillin/tazobactam; TIM, Ticarcillin/clavulanic acid; CIP, ciprofloxacin; CEF, cefsulodin; AZT, aztreonam; TOB, tobramycin; TOB/CAZ+A, tobramycin and ceftazidime with alafosfalin; TOB/CAZ-A, tobramycin and ceftazidime